

#### **Restriction Enzymes**

Introduction

List of Isozymes

List of Restriction Enzymes

#### **Modifying Enzymes**

T4 DNA Ligase

**INSTANT Ligation Kit** 

T4 Polynucleotide Kinase (PNK)

Alkaline Phosphatase

#### **Nucleases**

Dnase I

DNase I (RNase Free)

RNase A (DNase Free)

#### Other Enzymes & Proteins

Recombinant RNase Inhibitors

**Proteases** 

#### **Products for PCR**

Introduction on GeNei Taq DNA Polymerase

Products for PCR/RT PCR

Tag DNA Polymerase

Taq DNA Polymerase Buffers

PCR Master Mix (2X)

GeNei<sup>™</sup> SYBR Green qPCR Master Mix (2X)

Plant Direct GenAmp PCR Kit

GeNei™ DNA Amplification Reagent Kit

GenAmp Direct PCR Kit (from Mammalian cell lines, Bacteria and Yeast)

Mouse Tissue Direct GenAmp PCR Kit

Red Taq DNA Polymerase

Red Dye PCR Master Mix Kit (2X)

HotStart Taq DNA Polymerase

GeNei<sup>™</sup>HotStart PCR Master Mix

Effi-Taq™ DNA Polymerase

PR Polymerase

XT-5 PCR System

XT-20 PCR System

Bst Polymerase

MAGIC Amplification Solution (2.5X)

RAPD Primer Set

dNTPs mix, Set and Solution

Mineral Oil

#### **DNA Diagnosis and Reagents**

White Spot Syndrome Virus (WSSV) Detection Kit

GeNei<sup>™</sup> Amplification Reagents Set for Malarial Parasites

GeNei<sup>™</sup> Amplification Reagents Set for Human Papilloma

GeNei<sup>™</sup> Amplification Reagents Set (for Mycobacterium tuberculosis)

#### **Products for RT-PCR**

M-MuLV Reverse Transcriptase

MMLV III Reverse Transcriptase

M-MuLV RT PCR Kit

RT III RT-PCR Kit

One Step M-mLV RT-PCR Kit

cDNAdirect<sup>™</sup> Kit

Random Hexamer

Oligo dT primer pd (T)

#### **Cloning and Expression**

pUC18

pUC 19

pBR322

GeNei<sup>™</sup> Instant Cloning Kit

GeNei<sup>™</sup> Competent Cells Preparation Kit (A),

50 reactions (Calcium Chloride method)

GeNei<sup>™</sup> Competent Cells Preparation Kit (B),

50 reactions (Modified Calcium Chloride method)

#### **Genomic DNA**

Calf Thymus DNA

Lambda DNA

B.subtilis Genomic DNA 119

E.coli Genomic DNA 119

Human Genomic DNA (from Blood cells) 119

Yeast Genomic DNA 119

#### **Bacterial Strains**

E.coli JM101

E.coli JM109

#### Mutagenesis

GeNei<sup>™</sup>InSite PCR-Based Site Directed Mutagenesis Kit

DNA Labelling Kit (by Nick Translation) 123



#### **Solution Based**

PureSol<sup>™</sup> Plasmid Isolation Kit

UniFlex<sup>™</sup> DNA Isolation Kit

GeNei<sup>™</sup> Plant DNA Extraction Kit (for PCR Amplification)

GeNei<sup>™</sup> CTAB Plant DNA Extraction Kit

GeNei<sup>™</sup> Whole Blood DNA Extraction Kit (from Fresh / Frozen Blood)

GeNei<sup>™</sup> Gel Extraction Kit, <sub>100</sub> preps

TriSol

STET Lysis Solution

#### **GeneiPureTM DNA Purification Kits**

GeneiPure<sup>™</sup> Plasmid Purification Kit

GeneiPure™ Bacterial DNA Purification Kit

GeneiPure<sup>™</sup> Yeast DNA Purification Kit

GeneiPure<sup>™</sup> Plant Genomic DNA Purification Kit

GeneiPure<sup>™</sup> Genomic DNA Purification Kit- Mammalian Tissues

GeneiPure™ Genomic DNA Purification Kit- Cells and Blood

GeneiPure<sup>™</sup> Quick PCR Purification Kit

GeneiPure<sup>™</sup> Gel Extraction Kit

GeneiPure<sup>™</sup> Fungal Genomic DNA Purification Kit

gDNA Purification Kit- Cotton Leaves, Seed and Lint

#### **GeneiPureIDTM DNA Isolation Kits**

GeneiPureID<sup>™</sup> DNA Isolation Kit- Dried Blood

GeneiPureID<sup>™</sup> DNA Isolation Kit for Semen

GeneiPureID<sup>™</sup> DNA Isolation Kit- Bone

GeneiPureID<sup>™</sup> DNA Isolation Kit- Saliva

GeneiPureID<sup>™</sup> DNA Isolation Kit- Skin

#### **Products for RNA**

RaFlex<sup>™</sup> Total RNA Isolation Kit (for Plants)

RaFlex<sup>™</sup> Total RNA Isolation Kit

(for Bacteria, Blood, Animal Cells & Tissues)

Plant RNA Isolation Kit

#### GeneiPure™ Total RNA Isolation Kits

GeneiPure<sup>™</sup> Total RNA Isolation Kit- Bacteria

MicroRNA Isolation Kit- Cells & Tissues

Total RNA Isolation Kit- Blood

GeneiPure<sup>™</sup> Total RNA Isolation Kit- Yeast

GeneiPure<sup>™</sup> Total RNA Isolation Mini Kit – Plants



#### GenPro™ 3-in-1 Isolation Kits

GenPro<sup>™</sup> 3-in-1 Isolation Kit- Cells and Tissues

GenPro<sup>™</sup> 3-in-1 Isolation Kit- Blood

GenPro<sup>™</sup> 3-in-1 Isolation Kit- Plant

GenPro<sup>™</sup> 3-in-1 Isolation Kit- Bacteria

#### **Nuclease free Buffers**

Tris-Cl 1M (pH 8.0)

Saturated Phenol (Water) (Protease, DNase & RNase Free)

Saturated Phenol (Water)

50X Denhardt's Reagent

Saturated Phenol (Tris-HCl)

Lymphocyte Separating Solution

0.5M EDTA (DNase & RNase Free)

10X TE (DNase & RNase Free)

Sodium Acetate 3M (DNase & RNase Free)

Nuclease Free Water

3M Potassium Acetate, pH 5.5 (DNase & RNase Free)

#### **Agarose Gel Electrophoresis Reagents**

Agarose

StepUp<sup>™</sup> 50 bp DNA Ladder

StepUp<sup>™</sup> 100 bp DNA Ladder

StepUp<sup>™</sup> 250 bp DNA Ladder (100 Loads)

StepUp<sup>™</sup> 500 bp DNA Ladder

StepUp<sup>™</sup> 1 kb DNA Ladder

Supermix DNA Ladder

20 bp DNA Ruler

Low Range DNA Ruler (100bp - 3 kb)

Midium Range DNA Ruler (100bp - 5 kb)

High Range DNA Ruler (100bp - 10 kb)

Quantum<sup>™</sup> PCR Marker Low Range Ready to Use

Quantum<sup>™</sup> PCR Marker Medium Range Ready to Use

Lambda DNA / EcoR I Digest, Ready to Use

pBR322 DNA / Hae III Digest

pBR322 DNA / Msp I Digest

X174 / Hae III Digest

Lambda DNA / EcoR I Digest

Lambda DNA / Hind III Digest

**Proteases** 



#### Fine Chemicals, Reagents & Buffers

**IPTG** 

X-Gal

DTT (1,4-Dithiothreitol)

icoll-

Tris-Base

Tween-20

Guanidine Hydrochloride

CTAB Powder

#### **Products for Nucleic Acid Electrophoresis**

High Resolution Agarose

#### **Buffers for Electrophoresis**

50X TAE

10X TBE

**MOPS** Buffer

#### **DNA Gel Loading Buffers**

6X Gel Loading Buffer

6X Orange Gel Loading Buffer

6X Tris Gel Loading Buffer

NanoGreen DNA Elpho Buffer, 400 ml

6X Orange-G/ Cresol Red DNA Gel Loading Buffer

6X Cresol Red DNA Gel Loading Buffer

Nucleic Acid Agarose Gel Electrophoresis Kit

DNA Silver Staining Kit

# TOOL FOR GENOMIC RESEARCH

# **Restriction enzymes**

Genei's restriction enzymes are thoughtfully provided in convenient pack sizes and optimal concentrations, with a specific emphasis on offering high concentrations for efficient genomic DNA digestions. Each enzyme lot undergoes rigorous scrutiny to ensure both its integrity and functional purity throughout and after the manufacturing process. Regular assays for enzyme activity are conducted quarterly, further affirming the quality and reliability of our products.

Stability checks have demonstrated that most of these enzymes maintain their effectiveness even when stored at -20°C for over a year. This longevity is particularly advantageous for concentrated enzymes, which tend to retain their activity for extended durations compared to their more diluted counterparts.

For user convenience, our restriction enzymes are categorized into five groups based on assay conditions, with color-coded enzyme and buffer vials for easy identification. Additionally, we provide a 10X assay buffer and 100X nuclease-free BSA (wherever required) with the enzyme, all supplied at no extra cost. Should you have additional requirements for reaction buffers, our catalogue includes a reference index for your convenience.

# Quality Checks: >

The catalytic activity of our offered restriction enzymes is gauged by determining the minimum quantity of enzyme needed to generate the enzyme-specific final fragment pattern of  $\lambda$  DNA, which holds true in most instances.

#### Unit Definition:

Our unit definition is standardized: one unit is the quantity of enzyme necessary to achieve a complete digest of 1  $\mu$ g of lambda DNA in a reaction volume of 50  $\mu$ l over 60 minutes. This measurement is conducted under optimal conditions of salt

concentration, pH, and temperature. This stringent and well-defined unit definition allows for accurate and reproducible assessments of the enzyme's catalytic efficiency, ensuring reliable and consistent results for our users.

In some cases, the determination of the unit is based on digestion of  $\lambda$  dam- DNA, pBR 322 DNA, Ad2 DNA,  $\lambda$ / EcoRI digest or  $\lambda$ / Hind III digest.

# Overnight Nonspecific Nuclease Assay:

We employ an overnight non-specific nuclease assay as a rigorous quality control measure for each batch of our restriction enzymes. In this assay, different units of the enzyme are incubated with 1  $\mu$ g of substrate DNA under the recommended assay conditions, within a 50  $\mu$ l reaction volume, for a duration of 16 to 20 hours.

The assessment of the assay relies on the resulting banding pattern. A sharp, unaltered banding pattern is indicative of enzyme purity, demonstrating the absence of detectable non-specific nucleases. The certificate of analysis supplied with each enzyme includes information on the highest number of units that produced a sharp, unaltered pattern in this assay. This meticulous testing process ensures that our restriction enzymes meet the highest standards of quality and purity, providing researchers with reliable tools for their molecular biology experiments.

# Non-Specific Endonuclease assay:

The Non-Specific Endonuclease assay is specifically conducted for enzymes that lack recognition sites in the supercoiled plasmid substrate. In this test, the enzymes are incubated with 1  $\mu g$  of supercoiled (RFI form) DNA within a 0.05 ml reaction volume for a duration of 4 hours, adhering to the recommended assay conditions.

During the incubation period, a single non-specific nick in the supercoiled form results in the production of the nicked form (RF-II). These two distinct forms can be visually differentiated on an agarose gel. The assay allows for the estimation of

# GeNei TM

the percentage of conversion, providing valuable insights into the enzyme's behavior in the absence of specific recognition sites on the supercoiled plasmid substrate.

This meticulous assay contributes to our comprehensive quality control processes, ensuring that our enzymes meet the highest standards and perform reliably in diverse experimental conditions.

#### Ligation/Recut Assay: >

The Ligation/Recut Assay is employed to assess the integrity and purity of DNA fragments produced through a 3-100 fold excess of restriction enzyme digestion. In this assay, the DNA fragments are subjected to ligation. Subsequently, the ligated DNA fragments are recut using the same restriction enzyme.

An unaltered banding pattern observed after the recut process serves as an indication of intact 5' and 3' termini, as well as the absence of contaminating nucleases. The success of this assay is determined by the estimated levels of ligation and re-cleavage, both of which must exceed 70-90%. This stringent criterion ensures that the DNA fragments maintain their structural integrity and that the restriction enzyme used is free from contaminants that could compromise the accuracy of downstream applications.

# Blue/White Standard Assay:

The Blue/White Standard Assay is a highly sensitive method employed for evaluating restriction enzymes used in cloning applications. This assay enables the detection of low levels of nuclease contamination within restriction enzymes, specifically those with recognition sites in the multiple cloning regions within the Lac  $Z\alpha$  gene of the plasmid.

In this assay, the plasmid is cleaved with a 3-50 fold excess of the enzyme. The cleaved DNA is then ligated and used to transform competent Dh5 $\alpha$  E. coli cells. The transformed cells are cultured on a selective medium to ensure that all growing colonies originate from E. coli cells transformed with the ligated vector. These colonies are subsequently tested for the integrity of the

polylinker site by examining the presence of the functional  $\beta$ -galactosidase locus. Only colonies that turn blue upon addition of X-gal and IPTG have an intact  $\beta$ -galactosidase locus, indicating an unchanged polylinker site within this structural gene due to cut and re-ligation.

For enzymes generating sticky ends, the permissible percentage of white colonies is up to 2%, while for enzymes generating blunt ends, the acceptable percentage is up to 10%. This assay offers a stringent evaluation of the restriction enzymes, ensuring their suitability for precise cloning applications.

#### Star activity:

Star activity occurs when a restriction enzyme displays non-specific cleavage at sites other than its canonical recognition sequence, especially under conditions that deviate from the optimal. The recommendation for careful use serves to prevent unintended cleavage and ensures the reliability of experimental outcomes when employing these specific enzymes.

Regarding star activity, it's crucial to exercise caution and adhere to precise assay conditions when using certain enzymes. Genei specifically recommends careful handling of a subset of enzymes that are particularly sensitive to variations in conditions. Enzymes prone to star activity under conditions of high units per microgram of DNA and/or prolonged incubation periods include BamH I, EcoR I, Kpn I, Nco I, Hinf I, PvuII, Sau3A I, Ssp I, Sal I, Nhe I, and Kpn I.

# Factors Influencing Restriction Enzyme Activity

#### Nature of DNA:

The nature of the substrate strongly influences the activity of restriction enzymes. The most important parameters are:

- Base distribution in natural DNA
- ◆ Tertiary structure of DNA
- Base composition of the flanking sequence
- The position of the cleavage site with respect to each other.

The nature of the substrate strongly influences the activity of restriction enzymes. The most important parameters are:

#### Temperature:

Temperature is a critical factor influencing the efficiency of DNA digestion, and the optimal temperature varies across different restriction enzymes. Genei provides a range of restriction enzymes, some of which have optimum incubation temperatures differing from the standard 37°C. The enzymes falling into this category are listed below. It's important to be aware of and adhere to the recommended temperature conditions for each specific enzyme to achieve optimal results in DNA digestion experiments.

Enzymes	Assay Temperature
Taq I	65° C

Tris Hcl is the most commonly used buffering agent in reaction mixtures. The buffer system is temperature dependent, the change in pH per 10°C amounts to approx. 0.3. The optimal activity of restriction enzymes is significantly influenced by the appropriate ionic environment. Magnesium ions (Mg2+) are an absolute requirement for all restriction enzymes. The addition of other salt components, however, varies among different nucleases.

Moreover, the presence of Bovine Serum Albumin (BSA) in the reaction mix can play a crucial role in enzyme activity. BSA serves multiple functions, including enzyme stabilization, binding of certain

impurities, and prevention of enzyme adsorption to the surfaces of test tubes. These factors underscore the importance of carefully considering and adjusting the ionic conditions and the inclusion of BSA to ensure the optimal performance of restriction enzymes in experimental setups.

#### Methylation of DNA:

Restriction endonucleases are part of prokaryotic restriction/modification systems. The digestion of DNA isolated during cloning steps in bacterial cells can be strongly affected by the methylation of specific adenosine or cytosine residues in the recognition sequence of the restriction enzyme of interest.

Many E.coli host strains possess two nucleotide sequence specific methylases; the dam methylase which modifies adenine residues to  $N^6$ -methyladenine in the sequence GATC and the dcm methylase which modifies the internal cytosine residues to 5-methylcytosine in CCAGG or CCTGG sequences.

Restriction enzymes affected by dam and dcm methylation are listed below.

Enzymes	inhibited	Enzymes inhibited bydcm methylation
Mbo I	+ /GATC	+ Stu I AGG/CCT
Taq I	+ T/CGA	
Xba I	+ T/CTAGa	

CpG Methylation is found in higher eukaryotes. These enzymes (e.g. Dnmt1) transfer a methyl group to the C5 position of cytosine residues. Patterns of CpG methylation are heritable, tissue specific, and correlate with gene expression. Consequently CpG methylation has been postulated to play a role in differentiation and gene expression.

This CpG methylation patterns will not be retained once the DNA is cloned into a bacterial host.

Genei Restriction Enzymes inhibited by CpG methyltion are listed below.

Enzymes inhibited by	CpG methylation
SalI	G/TCGAC



# **Reaction Buffer for Restriction Enzymes**

Genei provides colour coded10X assay buffer with each restriction enzyme to ensure optimal activity. Some restriction enzymes require BSA at a final concentration of 100  $\mu$ g/ml for optimal activity. BSA is supplied as 10 mg/ml (100 X) stock when required and should be added to the reaction mixture.

## Final Concentration in mM (1X Recipe)

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Buffer	Tris HCl	Tris - Acetate	Sodium Chloride	Magnesium Chloride	Magnesium Acetate	Potassium Acetate	Potassium Chloride	DTT	рН	Enzymes
А	10	-	150	7	-	-	-	1	7.9	EcoR V, Not I*, Sal I
В	10	-	100	10	-	-	-	1	8.0	Bgl I, Bgl II, Hinc II, Mlu I, Ssp I.
С	1 0	-	50	10	-	-	-	1	7.8	Alu I, Hae III, Hinf I, Msp I, Nhe I, Pvu II, Stu I, Xba I.
D	Optimised Conc	_	-	10	_	_	Optimised Conc	-	Optimised Conc	BamH I, BstE II, EcoR I, Hind III, Mbo I, Mlu I, Nsi I, Taq I, Pst I, Pvu I, Spe I, Nco I, Nde I, Nru I
L	10	-	-	10	-	-	-	1	7.4	Hpa II, Sac I, Xma I
Е	1	33	-	-	10	66	-	0.5	7.9	Apa I, Ava I, Cla I, Dra I, Hpa I, Nae I, Sau3A I, SnaB I, Sfi I, Sma I,

#### Note

- 1. Not I\* Buffer A with 0.01% Triton X 100.
- 2. The enzymes printed bold need BSA for optimum activity.
- 3. Incase of Taq I, use unique buffer supplied



# **List of Isozymes**

Sl. No.	Restriction Enzymes	Sequence	Isozymes
1	EcoR V	GAT↓ATC	Eco32 I
2	Hae III	GG↓CC	Bsh I, BsuR I, Pal I
3	Kpn I	GGTAC↓C	Acc65 I, Asp718
4	Mbo I	<b>↓GATC</b>	BscF I, Bsp143 I, Dpn II, Kz09 I, Nde II, Sau3A I
5	Msp I	C↓CGG	Bsi S I, Hap II, Hpa II
6	Nco I	C↓CATGG	Bsp19 I
7	Nde I	CA↓TATG	FauND I
8	Not I	GC↓GGCCGC	CciN I
9	Sac I	GAGCT↓C	Ecl136 II, Eco ICR I, Psp124B I, Sst I
10	Spe I	A↓CTAGT	AclN I, Bcu I
11	Taq I	T↓CGA	TthHB8 I
12	Xho I	C↓TCGAG	PaeR7 I, Sfr274 I, Tli I

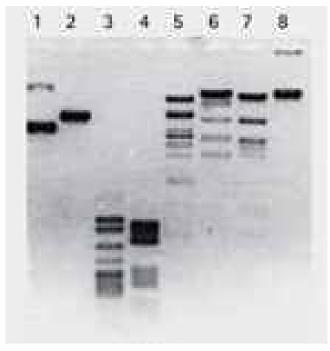
# Relative Activity of Restriction Enzymes in Genei Assay Buffer System

Sl. No.	Restriction Enzymes	10XAssay Buffers					
		Α	В	С	D	E	L
1	BamH I	25	50	50	100	50	50
2	EcoR I	25	50	50	100	50	0
3	EcoR V	100	75	50	100	50	0
4	Hae III	75	100	100	100	100	25
5	Hind III	0	0	25	100	25	0
6	Kpn I	0	0	75	25	50	100
7	Mbo I	50	75	50	100	50	0
8	Msp I	25	75	100	25	100	100
9	Nco I	0	50	50	100	50	50
10	Nde I	25	75	25	100	25	25
11	Not I	100	100	75	100	0	0
12	Pst I	50	50	50	100	25	50
13	Pvu I	0	25	100	75	50	25
14	Sac I	25	25	50	50	100	100
15	Sal I	100	50	0	25	0	0
16	Spe I	25	25	50	100	50	50
17	Ssp I	25	100	100	100	100	100
18	Taq I	25	75	25	25	50	25
19	Xba I	0	0	100	50	100	0
20	Xho I	100	100	100	100	75	25

#### Note

- $\textbf{1.} \quad \text{Supplies the buffers that are typed in italics and bold with the respective enzymes}.$
- 2. Chart serves as a guide to choose the compatible buffer for double digestion.
- $\textbf{3.} \quad \text{In case of Taq I, the percentage activity is reported with respect to the unique buffer supplied.}$

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Lane 1: pBR322 uncut

Lane 2: pBR322 cut with Bam HI
Lane 3: pBR322 cut with Msp I
Lane 4: pBR322 cut with Hae III
Lane 5: \( \Lambda \) DNA cut with Nde
Lane 7: \( \Lambda \) DNA cut with Hind III

Lane 8: Uncut & DNA

Fig. 1: 1μg each of pBR322 plasmid and Λ phage DNA were digested with Restriction Enzymes and electrophoresed on 1.2% agarose gel.

#### References:

- Roberts RJ (April 2005). "How restriction enzymes became the workhorses of molecular biology". Proceedings of the National Academy of Sciences of the United States of America. 102 (17)
- Roberts RJ, Vincze T, Posfai J, Macelis D (January 2007). "REBASEenzymes and genes for DNA restriction and modification". Nucleic Acids Research.
- Primrose SB, Old RW (1994). Principles of gene manipulation: an introduction to genetic engineering. Oxford: Blackwell Scientific. ISBN 0-632-03712-1.
- Micklos DA, Bloom MV, Freyer GA (1996). Laboratory DNA science: an introduction to recombinant DNA techniques and methods of genome analysis. Menlo Park, Calif: Benjamin/Cummings Pub. Co.
- Massey A, Kreuzer H (2001). Recombinant DNA and Biotechnology: A Guide for Students. Washington, D.C: ASM Press.

# Applications:

 DNA Cloning: Restriction enzymes are widely used in DNA cloning procedures. They cleave both plasmid vectors and foreign DNA at specific recognition sites, allowing researchers to insert foreign DNA into the vector for the creation of recombinant DNA.

- Construction of Recombinant DNA Molecules: Restriction enzymes facilitate the construction of recombinant DNA molecules by cutting DNA at specific sites. This is crucial for the creation of genetically engineered organisms, gene therapy vectors, and the production of recombinant proteins.
- Gene Mapping: Restriction enzymes are employed in genetic mapping studies to identify and map specific DNA sequences. By digesting genomic DNA with various restriction enzymes, researchers can create a restriction map that shows the positions of recognition sites.
- Southern Blotting: Restriction enzymes are used in Southern blotting to digest genomic DNA, generating fragments that can be separated by gel electrophoresis. These fragments are then transferred to a membrane and probed with labelled DNA or RNA to detect specific sequences.
- DNA Fingerprinting: Restriction fragment length polymorphism (RFLP) analysis, which relies on the variations in DNA fragment sizes generated by restriction enzymes, is used in DNA fingerprinting for genetic identification and paternity testing.
- Polymerase Chain Reaction (PCR) Analysis: Some applications of PCR involve the use of restriction enzymes. For example, after amplifying a specific DNA region, researchers may use a restriction enzyme to analyze polymorphisms or specific mutations in the amplified product.
- Site-Directed Mutagenesis: Restriction enzymes are utilized in site-directed mutagenesis to introduce specific changes into a DNA sequence. After introducing a mutation, the DNA can be ligated back together, and the altered gene can be expressed or studied.
- Gene Expression Studies: By using restriction enzymes to digest genomic DNA, researchers can study the structure and organization of genes and regulatory elements. This is particularly relevant in studies of gene expression and regulation.
- Creation of DNA Fragments for Cloning Libraries:
   Restriction enzymes are used to generate defined
   DNA fragments from a larger genomic DNA



- sample. These fragments can be cloned into vectors to create genomic DNA libraries.
- Vector Preparation for DNA Sequencing: Restriction enzymes are employed to linearize plasmid vectors for DNA sequencing. Linearized vectors serve as templates for DNA sequencing reactions.
- Molecular Diagnosis of Genetic Disorders:
   Restriction enzymes play a role in detecting
   specific genetic mutations associated with
   disorders. The digestion patterns obtained can
   aid in the diagnosis of genetic diseases.

 Golden Gate Cloning and Synthetic Biology: In synthetic biology, researchers use a modular cloning technique known as Golden Gate cloning, which involves the sequential use of type II restriction enzymes for the assembly of DNA fragments in a predetermined order.

#### Nature of DNA:

Inefficient excision of uracil from loop regions of DNA oligomers by E.coli uracil DNA glycosylase N.Vinay Kumar and U.Varshney\* Centre for Genetic Engineering, Indian Institute of Science, Bangalore 560012, India

Ordering Information.						
Cat. No	PI No.	Product Description \				
0100100021730	MBE1S	BamH I, 2000Units, 10 U/μl				
0100300021730	MBE3S	EcoR I, 4000Units, 20 U/μl				
0100300041730	MBE3L	EcoR I, 20000Units, 20 U/μl				
0100900021730	MBE9S	EcoR V, 1200Units, 10 U/μl				
0101000041730	MBE10L	Hae III, 4000Units, 10 U/μl				
0100600021730	MBE6S	Hind III, 4000Units, 20 U/μl				
0102200041730	MBE22L	<i>Kpn</i> Ι, 4000Units, 10 U/μl				
0102700041730	MBE27L	<i>Mbo</i> Ι, 400Units, 10 U/μl				
0103100021730	MBE31S	Msp I, 400Units, 10 U/μl				
0101500041730	MBE15L	Nco I, 400Units, 10 U/μl				
0106200021730	MBE62S	Nde I, 4000Units, 20 U/μl				
0101600041730	MBE16L	Not I, 400Units, 10 U/μl				
0101200021730	MBE12S	Pst I, 1200Units, 10 U/μl				
0101800041730	MBE18L	Sac I, 2000Units, 10 U/μl				
0100400041730	MBE4L	Sal I, 2000Units, 20 U/μl				
0100700021730	MBE7S	<i>Taq</i> I, 800Units, 10 U/μl				
0101100041730	MBE11L	Pvu II, 2000Units, 10 U/μl				
0104100041730	MBE41L	Ssp I, 500Units, 10 U/μl				
0102300021730	MBE23L	Xba Ι, 1000Units,10 U/μl				
0102400021730	MBE24S	Xho I, 1000Units, 10 U/μl				
0103800041730	MBE38L	SPE I, 400Units, 100U/ul				



## **MODIFYING ENZYMES**

#### **Unit definitions**

Unit definition varies from enzyme to enzyme and is given in the product description of this catalogue

#### **Performance Test**

Each enzyme is tested for its performance using tests that are mentioned in product description.

#### Assay for non-specific endonuclease

All modifying enzymes are checked for the absence of non-specific endonuclease activity by incubating excess of the enzyme with supercoiled plasmid DNA for several hours and determining the level of nicking in supercoiled form. The highest number of units producing unaltered pattern is reported on the data sheet supplied with the enzyme.

#### Assay for exonuclease activity

The absence of exonuclease activity is checked for all the enzymes by incubating the enzyme with lambda substrate for several hours and running on an agarose gel. The sharp pattern indicates the absence of exonuclease. The highest number of units producing unaltered pattern is reported on the data sheet supplied with the enzyme.

#### **Assay for RNase**

The absence of contaminating RNases is tested by incubating total RNA from HeLa cells with excess of enzyme for 4 hours at 37°C and checking for any degradation on gel.

#### **Purification**

All modifying enzymes are purified to near homogenity using the procedures developed at Genei.

#### Stability

All batches of modifying enzymes are routinely tested for stability by checking unit activity. Most of the enzymes are stable for more than 12 months when stored at -20°C. Exposure to temperatures greater than -20°C should be minimised whenever possible.

# **T4 DNA Ligase**

#### **Description:**

The search for an enzyme that could join DNA molecules had begun in earnest. The breakthrough came at the beginning of 1967, when Marty Gellert at the NIH showed that extracts of Escherichia coli could convert hydrogen-bonded circles of bacteriophage & DNA into a covalently closed, circular form. Within a few months, Gellert's and several other groups had independently purified an enzymatic activity that catalysed the formation of phosphodiester bonds between apposing 3' hydroxyl and 5' phosphoryl termini in a strand of DNA held in a double stranded configuration by Watson – Crick pairing.

#### Ligation of DNA is a three-step reaction

- (I) Formation of a covalent ligase-AMP intermediate,
- (ii) Transfer of the AMP to the 5' -phosphoryl terminus of DNA, and
- (iii) Attack on the AMP-DNA bond by the opposing 3' hydroxyl group the nick in the DNA is sealed and AMP is liberated (for review, see Pascal 2008).

These three sequential steps are carried out by three different ligase domains that form a catalytic region encircling the DNA substrate with each domain in contact with the DNA duplex. DNA ligases used in molecular cloning are either of bacterial origin or bacteriophage encoded. All eubacteria, whether thermophilic or mesophilic, contain a single ligase gene that encodes an NAD+ - dependent enzyme (Olivera and Lehman 1967; Takahashi et al. 1984). During the first step of a ligation reaction, the diphosphate linkage of NAD+ is used as a phosphor anhydride and the adenyl group is transferred to the '-amino group of a lysine residue in DNA ligase. The adenyl residue is then transferred to the 5' phosphoryl terminus of the DNA substrate, which becomes vulnerable to nucleophilic attack by the opposing 3' hydroxyl group. This results in the formation of a phosphodiester bond, elimination of AMP, and covalent joining of the DNA strands (for reviews, see Pascal 2008; Shuman 2009)

T4 DNA Ligase is used to covalently join DNA fragments with cohesive ends or blunt ends as well as repair single stranded nicks in ds DNA and RNA. The enzyme does not act on single stranded nucleic acids.

- Unit Definition: One Cohesive end ligation unit is defined as the amount of enzyme required to give approximately 50% ligation of 1 g DNA/Hind III digest in 30 minutes at 16°C in 20 l reaction mixture.
- Storage Buffer: T4 DNA supplied in 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol and 200g/ml Nuclease free BSA.
- Reagents Supplied with Enzyme: 10X Ligase Assay Buffer, and Dilution Buffer.
- ◆ 1 X Ligase Assay Buffer: 50mM Tris-HCl (pH 7.4 at 25°C), 10mM MgCl2, 20 mM DTT, 50 g/ml Nuclease free BSA and 1 mM ATP.
- Store: Store Enzyme Assay Buffers and Reagents at -20°C.

#### Note:

- Cohesive end ligation unit approximately equals 0.015 ATP-PP exchange unit (Weiss unit).
- Store buffer as aliquots to avoid freeze-thaw cycles.
- ✓ Carryout Cohesive End ligation at 16°C.
- ✓ Carryout Blunt End ligation at 25°C.

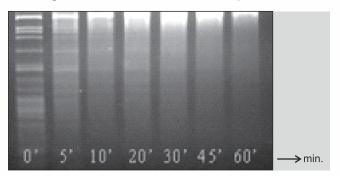
# Quality control assays: >

- ◆ Absence of Nuclease Activity: T4 DNA Ligase when incubated for 16 hours with 1 g of DNA digest at 37°C and 16°C showed sharp unaltered pattern on 1% agarose gel.
- ◆ Absence of Nickase Activity: T4 DNA Ligase when incubated for 16 hours with 1 g of supercoiled plasmid DNA at 37°C and 16°C showed unaltered pattern without nicking on 1% agarose gel.

#### Performance Test:

- Ligation-Recut Assay: T4 DNA Ligase is routinely used for quality checks of all cohesive and blunt end generating restriction enzyme in ligation recut assay.
- Blue-White Assay: T4 DNA Ligase is routinely used in Blue-White selection assays as quality checks for restriction enzymes.

 Cloning Assay: T4 DNA Ligase was used in cloning experiments by ligating cohesive and blunt ended inserts to suitable vectors. The clones thus generated were checked for expression.



Blunt End Ligation: Ligation of  $\emph{A}/\mbox{Hae}$  III blunt ended fragments using 1  $\mu\mbox{I}$  of T4 DNA ligase at 25° C at different time points from 0-60 minutes.

#### Applications: >

**DNA Cloning:** T4 DNA ligase is widely used in DNA cloning procedures to join DNA fragments with compatible cohesive ends (sticky ends). This is essential for creating recombinant DNA molecules and plasmids.

**Plasmid Ligation:** T4 DNA ligase is used to ligate foreign DNA fragments into plasmid vectors. This is a critical step in the construction of recombinant plasmids for gene cloning or expression studies.

**Gene Synthesis:** T4 DNA ligase can be employed in gene synthesis by ligating synthetic DNA oligonucleotides or DNA fragments to assemble a full-length gene.

**Site-Directed Mutagenesis:** T4 DNA ligase is used in site-directed mutagenesis protocols to ligate mutagenic oligonucleotides to a circular DNA template, introducing specific nucleotide changes in the target gene.

Adapter Ligation for Next-Generation Sequencing (NGS): T4 DNA ligase is used to ligate adapters to DNA fragments during library preparation for NGS, enabling the sequencing of DNA fragments with high-throughput technologies.

**Linker Ligation for DNA Amplification:** T4 DNA ligase is employed in linker ligation reactions, where specific DNA linkers are ligated to DNA fragments to facilitate subsequent PCR amplification.

# GeNei TM

RNA/DNA Chimeric Molecule Formation: T4 DNA ligase can be used to ligate RNA and DNA molecules together, allowing the creation of chimeric molecules for various applications, including RNA-protein interaction studies.

CircLigase-Independent Cloning (CIRCLE): T4 DNA ligase is utilized in some variations of the CircLigase-Independent Cloning (CIRCLE) method, which is used for the circularization and cloning of circular DNA molecules.

Library Construction for Nucleic Acid Selection: T4 DNA ligase is used in library construction for techniques like SELEX (Systematic Evolution of Ligands by Exponential Enrichment) to create diverse nucleic acid libraries.

Joining DNA Fragments for In Vitro Transcription Templates: T4 DNA ligase can be used to join DNA fragments in the construction of templates for in vitro transcription reactions.

#### Citation:

Intramolecular triplex potential sequence within a gene down regulates its expression in vivo Partha S.Sarkarl and Samir K.Brahmachari 2, \* Molecular Biophysics Unit and 2Centre for Genetic Engineering, Indian Institute of Science, Bangalore 560 012, India

#### References:

- A comprehensive approach for genome-wide efficiency profiling of DNA modifyingenzymes.
- Cell Reports Methods 28 March 2022- Charalampos Kyriakopoulos, Karl Nordström.Pascal Giehr.

# **Ordering Information:**

Cat. No	PI No.	Product Description
1100100021730	MME1S	T4 DNA Ligase, 8000Units, 400U/μl
1100100041730	MME1L	T4 DNA Ligase, 40000Units, 400U/μl
1160100021730	MME1HC	T4 DNA Ligase (High Conc.), 100000Units, 1000 U/μl

# **Instant Ligation Kit**

The INSTANT Ligation Kit is specially designed for efficient ligation of both cohesive and blunt ended DNA fragments in just 5 minutes at room temperature (25°C). The kit consists of INSTANT T4 DNA ligase and 2X INSTANT ligation buffer that significantly speeds up the process of ligation compared to overnight incubation. The ligated products can be directly used for transformation without any further purification steps.

This kit is supplied with reagents sufficient to perform 20 reactions.

#### Unique features:

- Ligation of both blunt and cohesive ends using same buffer.
- ◆ Incubation for just 60 minutes at room temperature (25°C)
- No additional purification steps required prior to transformation.

#### Kit Contents:

- Instant T4 DNA Ligase
- ◆ 2X INSTANT Ligation buffer
- Instruction Manual

Storage: -20°C

Buffer set for Ligation –

Dilution buffer for T4 DNA ligase, 0.5 ml X 2

## Applications: >

- DNA Cloning: Ligation kits are extensively used in DNA cloning procedures. They facilitate the joining of DNA fragments with compatible cohesive ends (sticky ends) or blunt ends, allowing the creation of recombinant DNA molecules.
- Plasmid Construction: Ligation kits are employed to ligate foreign DNA fragments into plasmid vectors, an essential step in constructing recombinant plasmids for gene cloning or expression studies.

- Gene Synthesis: Ligation kits are used in gene synthesis protocols to ligate synthetic DNA fragments or oligonucleotides, assembling a fulllength gene.
- Adapter Ligation for Next-Generation Sequencing (NGS): Ligation kits are used in library preparation for NGS. Adapters containing specific sequences are ligated to DNA fragments, allowing them to be sequenced using highthroughput technologies.
- Linker Ligation for PCR Amplification: Ligation kits can be used to ligate specific DNA linkers to DNA fragments, enabling subsequent PCR amplification for various applications, including the creation of DNA libraries.
- Site-Directed Mutagenesis: Ligation kits are used in site-directed mutagenesis protocols to ligate mutagenic oligonucleotides to circular DNA templates, introducing specific nucleotide changes in the target gene.
- RNA/DNA Chimeric Molecule Formation: Ligation kits may be used to ligate RNA and DNA molecules together, allowing the creation of chimeric molecules for applications such as RNA-protein I nteraction studies.
- Construction of Fusion Genes: Ligation kits can be used to join different DNA fragments, enabling the construction of fusion genes or chimeric proteins.
- DNA Concatenation: Ligation kits can be used to concatenate DNA fragments, allowing the

## References: >

- Lehman IR (November 1974). "DNA ligase: structure, mechanism, and function". Science. 186 (4166): 790
- Foster JB, Slonczewski J (2010). Microbiology: An Evolving Science (Second ed.). New York: W. W. Norton & Company.
- Yang Y, LiCata VJ (February 2018). "Pol I DNA polymerases stimulate DNA end-joining by Escherichia coli DNA ligase". Biochemical and Biophysical Research Communications. 497 (1): 13–18.

# **Ordering Information:**

Cat. No	PI No.	Product Description
1660300011730	KT91	GeNei™ Instant Ligation Kit, 20 reactions
1150400011730	MELB4	Buffer Set for Ligation - Dilution Buffer for T4 DNA Ligase, 0.5 ml x 2

# T4 polynucleotide kinase (PNK)

**Description:** T4 polynucleotide kinase (PNK), a member of the bifunctional enzyme family, exhibiting both 5'-kinase and 3'-phosphatase activities that play crucial roles in RNA and DNA repair processes. T4 PNK forms a homotetramer, featuring a C-terminal phosphatase domain and an N-terminal kinase domain.

The crystal structure of the isolated kinase domain, determined at 2.0 Å resolution, reveals a tunnel-like active site running through the center of the enzyme. This active site, with an entrance on the 5' OH acceptor side, is designed to accommodate a single-stranded polynucleotide.

**Unit Definition:** The unit of T4 polynucleotide kinase activity is defined as the amount of enzyme capable of catalysing the production of one nanomole (nmol) of acid-insoluble ^32p in 30-minute incubation period at 37°C.

Assay Buffer (1X): 70 mM Tris-HCl (pH 7.6), 10 mM  $MgCl_2$  and 5 mM DTT. Buffer supplied at 10X concentration.

**Storage Buffer:** 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 10 mM 2- Mercaptoethanol, 50 mM KCl, 50% Glycerol and 200 µg/ml Nuclease free BSA.

# Quality control assays:

- Absence of Nuclease Activity: T4 Polynucleotide Kinase when incubated for 16 hour with 1 g of DNA digest at 37°C showed sharp unaltered pattern on 1% agarose gel.
- Absence of Nickase Activity: T4 Polynucleotide Kinase when incubated for 16 hour with 1 g of supercoiled plasmid DNA at 37°C showed unaltered pattern without nicking on 1% agarose gel.
- Absence of Phosphatase Activity: T4
   Polynucleotide Kinase when incubated for 30 minutes at 37°C with suitable substrate was determined to be free of phosphatase contamination.

# GeNei TM

Performance Test: Dephosphorylated pUC18
 DNA/EcoR I digest was kinased, ligated and transformed into E.coli host along with proper controls and kination efficiency was determined.

#### Applications: >

- 5' End Labeling of DNA and RNA: T4 PNK catalyzes the transfer of a phosphate group to the 5' hydroxyl terminus of DNA or RNA molecules. This makes it valuable for labeling the 5' ends of nucleic acids with radioactive or non-radioactive markers, facilitating downstream applications like DNA sequencing, Northern blotting, or primer extension assays.
- Blunting DNA Ends: T4 PNK can be used to fill in or blunt DNA ends by phosphorylating the 5' terminus of one strand and removing the 3'phosphate from the other strand. Blunting is often required for subsequent DNA ligation reactions.
- 3' End Labeling of DNA: T4 PNK can be utilized to label the 3' ends of DNA by transferring a phosphate group to the 3' hydroxyl terminus. This labeled DNA can be used in applications such as DNA sequencing or hybridization studies.
- Repair of DNA Nicks: T4 PNK can repair singlestranded nicks in DNA molecules by phosphorylating the 5' terminus of the nicked strand. This is important in DNA repair processes.
- Preparation of Cloning Vectors: T4 PNK is used to treat linearized DNA vectors by phosphorylating their ends. This ensures that the vector ends are compatible for ligation with insert DNA fragments during cloning procedures.
- RNA 5' End Labelling: T4 PNK can be used to label the 5' ends of RNA molZecules, which is useful in studies involving RNA-protein interactions, RNA localization, or RNA turnover.
- Preparation of Kinase-Dead Substrates: T4 PNK
   can be used to generate kinase-dead versions of
   substrates for experiments where
   phosphorylation is undesirable or needs to be
   controlled.
- Treatment of DNA for Kinase-Independent

- **Ligations:** T4 PNK treatment can be part of a protocol to prepare DNA for ligation reactions, particularly in situations where kinase-independent ligations are desired.
- Labelling Oligonucleotide Probes: T4 PNK is commonly used to label oligonucleotide probes for hybridization-based assays, such as Southern or Northern blotting.
- Preparation of Radiolabelled ATP: T4 PNK can be used to radiolabel ATP for use in various enzymatic assays.

#### Citations:

- Angle and locus of the bend induced by the MspI DNA methyltransferase in a sequence-specific complex with DNA Ashok K. Dubey\* and Sanjoy K. Bhattacharya Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology-Delhi, Hauz Khas, New Delhi-110016, India
- Hairpin duplex equilibrium reacted in the A®B transition in an undecamer quasi-palindrome present in the locus control region of the human b-globin gene cluster Mahima Kaushik, Ritushree Kukreti1, Deepak Grover1, Samir K. Brahmachari1 and Shrikant Kukreti\* Department of Chemistry, University of Delhi (North Campus), Delhi 110007, India and 1 Institute of Genomics and Integrative Biology (CSIR), Delhi University Campus, Delhi 110007, India
- Intramolecular triplex potential sequence within a gene down regulates its expression in vivo Partha S.Sarkarl and Samir K.Brahmachari 2, \* Molecular Biophysics Unit and 2Centre for Genetic Engineering, Indian Institute of Science, Bangalore 560 012, India

#### References:

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC126130/
- Developmentally Regulated Dual-Specificity Kinase from Peanut That Is
   Induced by Abiotic Stresses1 Parvathi Rudrabhatla and Ram
   Rajasekharan\* Department of Biochemistry, Indian Institute of Science,
   Bangalore 560012. India



#### **Ordering Information:**

Cat. No	PI No.	Product Description
1100400051730	MME4L	. T4 Polynucleotide Kinase, 1000Units, 10U/µl

# **Alkaline Phosphatase**

#### Description:

Alkaline phosphatase (ALP) is an enzyme classified as a hydrolase, specifically functioning as a phosphatase. Its primary role involves the removal of phosphate groups from a variety of substrates, including proteins and nucleotides. This dephosphorylation activity is integral to various biological processes and analytical techniques in molecular biology, where ALP is frequently employed for dephosphorylating DNA or RNA to prevent self-ligation during cloning procedures, among other applications.

#### **Unit Definition:**

One unit is defined as the amount of enzyme that hydrolyzes 1 micromole of p-nitrophenyl phosphate to p-nitrophenol in 1 minute at 37° C in a volume of 1 milliliter.

#### Assay buffer (1X):

50 mM NaCl, 10 mM Tris-HCl (pH 7.9), 10 mM MgCl<sub>2</sub> and 1 mM DTT. Buffer Supplied at 10X concentration.

Storage buffer: 50 mM KCl, 10 mM Tris-HCl (pH 8.2), 1 mM MgCl<sub>2</sub>, 0.1 mM ZnCl<sub>2</sub> and 50% glycerol.

# Quality control assays:

- Absence of Nuclease Activity: Alkaline Phosphatase when incubated for 16 hours with 1g of DNA digest at 37°C showed sharp unaltered pattern on 1% agarose gel.
- Absence of Nickase Activity: Alkaline Phosphatase when incubated for 16 hours with 1g of supercoiled plasmid DNA at 37°C showed unaltered pattern without nicking on 1% agarose gel.

 Absence of Ribonuclease: Alkaline Phosphatase when incubated for 4 hours with 1 g E.coli RNA at 37°C showed no degradation of RNA as visualized on agarose gel.

#### Performance Test: >

 pUC18 DNA/EcoR I digest was dephosphorylated, kinased ligated and transformed into *E.coli* host along with proper controls and dephosphorylation efficiency was determined.

Storage: -20°C

#### Applications: >

- Dephosphorylation of Vector DNA: CIP is used to dephosphorylate the 5' ends of linearized vector DNA. This prevents the vector from self-ligating and ensures that only DNA inserts with compatible ends can be ligated into the vector.
- Dephosphorylation of PCR Products: CIP treatment of PCR products can be employed to remove 5' phosphate groups. This is useful when unincorporated primers or nucleotides with 5' phosphates could interfere with downstream applications.
- RNA 5' Dephosphorylation: CIP can be used to dephosphorylate the 5' ends of RNA molecules, which is beneficial in certain applications such as RNA dephosphorylation prior to ligation reactions or in studies of RNA structure.
- Removal of 5' Phosphates for Kinase-Dead Substrates: CIP can be used to generate kinase-dead versions of substrates for experiments where phosphorylation is undesirable or needs to be controlled.
- Dephosphorylation of Oligonucleotides: CIP is used to dephosphorylate the 5' ends of oligonucleotides, especially when preparing probes for labeling or in studies requiring modified oligonucleotides.
- Dephosphorylation of Restriction Enzyme-Digested DNA: CIP treatment of DNA fragments generated by restriction enzyme digestion can be used to remove 5' phosphates, preventing the



- self-ligation of fragments, and facilitating subsequent DNA manipulations.
- Preparation of Vector for Blunt-End Cloning: CIP treatment is employed in the preparation of vectors for blunt-end cloning. It dephosphorylates the 5' ends of the linearized vector to prevent self-ligation and promote the ligation of blunt-ended inserts.
- Treatment of DNA for Kinase-Independent Ligations: CIP treatment can be part of a protocol to prepare DNA for ligation reactions, particularly in situations where kinase-independent ligations are desired.
- Removal of 5' Phosphates in DNA-Protein Crosslinks: CIP treatment can be used in experiments involving DNA-protein crosslinks, helping to remove 5' phosphates and modify DNA ends for further analyses.

 End-Repair of DNA Fragments: CIP can be used as part of an end-repair process for DNA fragments, preparing them for subsequent applications such as cloning or library preparation.

#### References:

- Schlesinger MJ, Barrett K (November 1965). "The reversible dissociation of the alkaline phosphatase of Escherichia coli. I. Formation and reactivation of subunits
- A Histochemical Procedure for Localizing and Evaluating Leukocyte Alkaline Phosphatase Activity in Smears of Blood and Marrow-LEONARD
- Yeh MF, Trela JM (May 1976). "Purification and characterization of a repressible alkaline phosphatase fromThermus aquaticus". The Journal of Biological Chemistry.

Cat. No	PI No.	Product Description
1100800031730		Alkaline Phosphatase (CIP) (Molecular Biology

#### **NUCLEASES**

# **DNase I**

DNase I an endonuclease of the DNase family coded by the human gene DNASE  $I^{\scriptscriptstyle{[5]}}$ . DNase I is a nuclease that cleaves DNA preferentially at phosphodiester linkages adjacent to a pyrimidine nucleotide, yielding 5'-phosphate-terminated polynucleotides with a free hydroxyl group on position 3', on average producing tetranucleotides. It acts on single-stranded DNA, double-stranded DNA, and chromatin. In addition to its role as a wastemanagement endonuclease, it has been suggested to be one of the deoxyribonucleases responsible for DNA fragmentation during apoptosis.

Deoxyribonuclease, I catalyses random degradation of both single and double-stranded DNA to produce 5'-P terminal oligonucleotides. The source of this enzyme is from the Bovine pancreas .The enzyme loses its activity irreversibly by heat treatment at 80°C for 10 minutes. DNase I is also inhibited by EDTA, EGTA and SDS and requires bivalent cations for maximal activity. Supplied as white lyophilised powder which dissolves readily at 5 mg/ml in 0.15 M sodium chloride/glass distilled water to give a clear colourless solution.

## Features : >

- Unit definition: One unit is that amount of enzyme causing an increase in absorbance at 260 nm by 0.001 per min. at 25° C and pH 5.0.
- Activity: ~ 600 units/mg (Kunitz)
- Appearance : White Crystalline powder.

Storage: -20°C

# Applications: >

- Deoxyribonuclease I (bovine pancreas) is used to catalyze random degradation of both single and double-stranded DNA producing 5'-P terminal oligonucleotides.
- Cell lysis- to remove the viscosity caused by the DNA content in bacterial cell lysates or to remove the DNA templates from RNAs produced by in vitro transcription.

• DNase is often included in tissue dissociation protocols to digest DNA that has leaked into the dissociation medium because of cell damage.

#### References:

- Junowicz, E. (1973). "Studies on bovine pancreatic deoxyribonuclease A.
   II. The effect of different bivalent metals on the specificity of degradation of DNA". Biochim. Biophys. Acta.
- Ohkouchi, S.; Shibata, M; Sasaki, M; Koike, M; Safig, P; Peters, C; Nagata, S; Uchiyama, Y (2013). "Biogenesis and proteolytic processing of lysosomal DNase II"
- Suck, D.; Oefner, C.; Kabsch, W. (1984). "Three-dimensional structure of bovine pancreatic DNase I at 2.5 A resolution". The EMBO Journal.

#### **Ordering Information:**

Cat. No	PI No.	Product Description
2150280011730	FC28B	DNase I, 1gm
2150280501730	FC28L	DNase I, 50mg

# **DNase I (RNase Free)**

DNase I (bovine pancreas) is a glycoprotein and double strand specific endonuclease which is made free of RNase activity. It is best suited for specific applications, where maintenance of RNA integrity is critical. The protein requires divalent cations for maximal activity.

**Unit Definition :** One unit is that amount of enzyme required to digest 1  $\mu g$  of plasmid DNA to oligonucleotides in 10 mins. at 37° C.

## Quality control assays:

- Absence of RNase contamination: Incubation of RNA transcript with DNase I Ribonuclease not deteactable
- Performance Test: One unit of DNase I completely degrades 1ug of plasmid DNA in 10 Minutes at 37°C

## **Applications:**

 DNase I (RNase free) is used in purification of DNA- free RNA made by in-vitro synthesis using SP6 or T7 RNA Polymerase system.

# **GeNei** TM

- Used for radioactive labeling by nick translation.
- To remove traces of DNA during RNA isolation prior to RT-PCR(in-vitro transcription)
- Clinical Applications: Human DNase I exhibits polymorphism that can be used for forensic identification and for correlation with certain diseases. Variations in serum DNase activities have been implicated as the result of disease states and measurements of DNase activities are often used for diagnosis and prognosis. Recombinant human DNase I has been administered in cystic fibrosis patients to improve mucociliary clearance and pulmonary function.

Storage: -20°C.

#### References:

 Purification and characterization of deoxyribonuclease from small intestine of camel Camelus dromedarius-Somia S. Abdel-Gany,a Mohamed O. El-Badry,a Afaf S. Fahmy, a and Saleh A. Mohamed<sup>a,a,\*</sup>

### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
0655600011730	FC56	DNase I (RNase Free),
		1000Units

# RNase A (DNase free)

# Description:

Bovine pancreatic ribonuclease A belongs to the superfamily of pancreatic ribonucleases. It functions as an endo phosphodiesterase, hydrolyzing the internal phosphate linkage of single-stranded RNA into its constituent nucleotides. This enzyme exhibits specificity for cleaving at the 3'-end of a C or U residue, with the 5' side being either a pyrimidine or purine residue, resulting in the formation of a 3'-phosphorylated C or U product.

Storage: -20°C

#### **Related Product:**

RNase A (DNase Free)

#### Quality Assays: >

#### Absence of DNase contamination:

- Final concentration of 100-200µg/ul is free of nickase and at 37°C for 4 hours
- Nuclease

#### Performance Test:

 1 μg of plasmid DNA isolated by alkaline lysis method was incubated with RNase A, at a final concentration of 20 μg/ml, for 30 minutes at 37°C in a 50 μl reaction volume. Sample DNA then loaded on a 1% agarose gel along with controls showed >90% degradation of RNA.

#### Applications:

- RNA Purification: RNase A is used to degrade RNA contaminants in DNA preparations. It is often added to DNA extraction or purification protocols to remove RNA, ensuring the purity of the isolated DNA.
- RNA Digestion in Protein Purification: In protein purification processes, particularly when isolating nucleic acid-binding proteins, RNase A is added to remove RNA contaminants that may co-purify with the protein of interest.
- DNase I Inactivation: After DNase I treatment to remove DNA contamination from RNA samples, RNase A can be added to degrade the remaining DNase I and prevent it from interfering with downstream applications.
- RNA Cleanup in Molecular Biology Procedures:
   RNase A is used to clean up RNA samples by
   digesting residual RNA in molecular biology
   procedures. This is particularly important in
   experiments where removing traces of RNA is
   critical.
- Cell Culture Media Treatment: RNase A can be added to cell culture media to remove RNA released from lysed cells. This prevents RNA contamination in cell culture experiments, especially when studying RNA-dependent processes.
- Removal of RNA from Protein Preparations: In protein biochemistry, RNase A is added to protein preparations to remove co-purifying RNA

molecules. This step is essential for obtaining pure protein samples.

- Mapping RNA Secondary Structure: RNase A is used in RNA structure studies to cleave singlestranded regions. By examining the cleavage pattern, researchers can gain insights into the secondary structure of RNA molecules.
- RNA Labeling and Probing: RNase A is utilized in RNA labeling and probing experiments. It can be used to digest unbound or non-specifically bound RNA, leaving only the specifically labeled or probed RNA molecules.
- Removal of RNA in Protein-RNA Co-Immunoprecipitation (RNA-IP): In RNA-IP experiments where proteins associated with specific RNA molecules are immunoprecipitated, RNase A can be used to remove the RNA component, allowing the isolation of protein-RNA complexes for analysis.
- Analysis of RNA in Tissue Sections: RNase A treatment can be applied to tissue sections in situ to remove RNA, allowing researchers to

- specifically study protein localization or other features without interference from RNA.
- Preparation of RNA-Free DNA Templates: RNase A is used in the preparation of RNA-free DNA templates for various applications, such as PCR or DNA sequencing

#### References:

 Cuchillo CM, Nogués MV, Raines RT (September 2011). "Bovine pancreatic ribonuclease: fifty years of the first enzymatic reaction mechanism"

## **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
2150380501730	FC34S	RNase A, 50mg
2150382501730	FC34L	RNase A, 250mg
2150480101730	FC25S	RNaseA (DNase free), 10 mg
2150480501730	FC25L	RNaseA (DNase free), 50 mg
2150481001730	FC25J	RNaseA (DNase free), 100 mg

# **RECOMBINANT RNASE INHIBITOR**

# Description: >

Recombinant RNase Inhibitor is the engineered version of Human Placental RNase Inhibitor, produced through the cloning and expression of the complete open reading frame (ORF) of the gene in Escherichia coli (E. coli). This is the recombinant form of RNase inhibitor isolated from human placenta. The complete ORF of the gene is cloned and expressed in E. coli. The protein has a molecular weight of ~ 51 kDa. A minimum concentration of 1 mM/L dithiothreitol is required to maintain RNase inhibitor in a fully active form. RNase inhibitor inactivates RNase by binding non-covalently to the enzyme. The binding ratio of RNase inhibitor to RNase Ais 1:1.

**Unit Definition:** One unit is defined as the amount of RNase Inhibitor required to inhibit the activity of 5 nanograms of RNase A by 50%.

This standardized unit provides a measure of the inhibitory capacity of the RNase Inhibitor, specifically in terms of its effectiveness in preventing the activity of RNase A by half under specified conditions.

Storage buffer: 20 mM HEPES-KOH (pH 7.6), 50 mM KCl, 5 mM DTT and 50% glycerol.

**Note:** 5 mM DTT concentration is critical for RNase Inhibitor and so has to be maintained during long and repeated uses

## Quality control Assay:

- Absence of Nuclease Activity: RNase inhibitor when incubated for 16 hours with 1 g of DNA digest at 37°C showed sharp unaltered pattern on 1% agarose gel.
- Absence of Nickase Activity RNase inhibitor when incubated for 16 hours with 1 g of supercoiled plasmid DNA at 37°C showed unaltered pattern without nicking on 1% agarose gel.

# GeNei TM

 Absence of Residual RNase activity: RNase inhibitor when incubated with E.coli total RNA along with p-chloro mercuric benzoic acid (PCMB), 1X assay buffer and 50 mM DTT for 30 minutes at 37°C showed no degradation of RNA on 2% agarose gel. Note: PCMB inhibits RNase inhibitor activity.

#### Applications:

- RNA Isolation and Purification: RNase Inhibitor is added to RNA extraction and purification protocols to protect RNA from degradation by endogenous or exogenous RNases. It helps ensure the integrity of RNA samples during the isolation process.
- Reverse Transcription (cDNA Synthesis): RNase Inhibitor is commonly included in reverse transcription reactions to prevent degradation of RNA templates during the synthesis of complementary DNA (cDNA). This is crucial for obtaining accurate and representative cDNA for downstream applications.
- In Vitro Transcription: RNase Inhibitor is used invitro transcription reactions to protect RNA transcripts from degradation by RNases. This ensures the production of intact RNA molecules for various applications, such as RNA probes or in vitro translation studies.
- PCR with RNA Templates: When using RNA as a template in reverse transcription PCR (RT-PCR) or quantitative PCR (qPCR), RNase Inhibitor is added to prevent RNA degradation during the reaction, ensuring accurate and reliable amplification.
- RNA Storage: RNase Inhibitor is often included in RNA storage solutions to protect RNA samples from degradation during long-term storage. This is particularly important when working with precious or rare RNA samples.

## References:

- Kobe B, Deisenhofer J (Dec 1996). "Mechanism of ribonuclease inhibition by ribonuclease inhibitor protein based on the crystal structure of its complex with ribonuclease A". Journal of Molecular Biology. 264 (5): 1028–43
- Yakovlev GI, Mitkevich VA, Makarov AA (2006). "Ribonuclease inhibitors". Molecular Biology. 40 (6): 867–874

## **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
1101200011730	FC73S	Recombinant RNase inhibitor, 1000 Units, 10U/µl

#### **Proteases**

#### Proteinase K:

Proteinase K is a serine protease, and its primary function is to break down proteins by cleaving peptide bonds. It possesses a broad-spectrum proteolytic activity, allowing it to efficiently digest a wide array of proteins, including those that may prove resistant to other proteases.

In the fields of molecular biology and biochemistry, Proteinase K finds extensive utility. It serves as a valuable tool for the digestion of structural proteins and enzymes, facilitating the study and manipulation of biomolecules. One of its key advantages lies in its remarkable stability across a broad range of conditions, including variations in pH and temperature. Moreover, Proteinase K exhibits resilience to various detergents and denaturing agents, further enhancing its versatility.

Proteinase K is supplied in.

- Ready to use solution format with 50% glycerol and the powder is dissolved in a specially formulated storage buffer.
- Concentration supplied at 20mg/mL.
- Convenient and safe in handling
- Active in presence of SDS, EDTA, urea and has a pH optimum of 7.5 – 10.5.
- Nuclease free

## Applications: >

 Nucleic Acid Extraction: Proteinase K is often used in DNA and RNA extraction protocols to degrade and remove proteins that may co-purify with nucleic acids.



- RNA and DNA Digestion: Proteinase K can be used to digest RNA or DNA molecules in specific applications, such as in RNA or DNA mapping experiments or removing unwanted nucleic acid fragments from a sample.
- Protein Structure Studies: Proteinase K is employed in structural biology studies to cleave and remove specific protein domains or regions for crystallography and other structural analysis methods.
- Environmental Sample Processing: Proteinase K can be used in environmental studies to break down proteins in soil, water, or sediment samples, allowing for the analysis of microbial DNA or RNA.

#### References:

- https://en.wikipedia.org/wiki/Proteinase\_K
- https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/proteinase-k

#### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
2150581001730	PK2L	Proteinase K, Solution, 5 X1 ml (20 mg/ml)
2150180251730	PK1S	Proteinase K, 25 mg
2150181001730	PK1L	Proteinase K, 100 mg
2150100011730	PK1B	Proteinase K, 1 g

#### Storage:

- PK2 -20°C
- PK1 20°C

# **Products for PCR**

Polymerase Chain Reaction (PCR) is an effective tool to synthesize defined sequences of DNA using thermostable polymerases. PCR exploits the inherent stability of these enzymes at high temperatures to amplify about a million copies of desired DNA fragment using two specific oligonucleotide primers that anneal to opposite strands flanking the target DNA sequence to be amplified. PCR is now widely used in cloning and sequencing DNA fragments, as a diagnostic tool and to generate labelled probes. To cater to these varied applications, Genei has a range of PCR enzymes, buffers, kits and optimized protocols.

- 1. Taq DNA Polymerase: Taq DNA Polymerase, a thermostable enzyme used in most routine PCR amplifications for sizes ranging from 100 bp to 3 kb, is a highly pure enzyme that lacks 3'-5' exonuclease (proof reading) activity. The enzyme can also be used in applications like RAPD, RFLP and AFLP studies to detect variations within the genomes of species.
- **2. HotStart Taq DNA Polymerase:** HotStart Taq DNA Polymerase, used for improved PCR specificity, is an optimized mixture of highly purified Taq DNA

- Polymerase with high affinity anti-Taq monoclonal antibody that inhibits polymerase activity at suboptimal temperatures by binding to Taq DNA Polymerase.
- 3. Effi-Taq™ DNA Polymerase: Effi-Taq™ DNA Polymerase, a modified form of Taq DNA Polymerase is supplied in an inactive state that has no polymerase activity at ambient temperature. It is suitable for PCR systems with complex genomic templates, complex cDNA templates (RT-PCR), very low copy targets, multiple primers reaction (Multiplex PCR), high throughput PCR procedures and systems that are prone to form Primer Dimer.
- 4. PR Polymerase: PR Polymerase, used in PCR reactions that require high fidelity synthesis, is a thermostable enzyme that exhibits strong Proof Reading activity. The 3'-5' exonuclease mediated proofreading activity enables the enzyme to repair mis-incorporated nucleotides and reduce errors during polymerisation. This enzyme is ideal for high fidelity amplifications of short stretches of target DNA (< 1.5 kb). An improved and more defined range of long PCR enzymes



- RNA and DNA Digestion: Proteinase K can be used to digest RNA or DNA molecules in specific applications, such as in RNA or DNA mapping experiments or removing unwanted nucleic acid fragments from a sample.
- Protein Structure Studies: Proteinase K is employed in structural biology studies to cleave and remove specific protein domains or regions for crystallography and other structural analysis methods.
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#### References:

- https://en.wikipedia.org/wiki/Proteinase\_K
- https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/proteinase-k

#### **Ordering Information:**

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2150581001730	PK2L	Proteinase K, Solution, 5 X1 ml (20 mg/ml)
2150180251730	PK1S	Proteinase K, 25 mg
2150181001730	PK1L	Proteinase K, 100 mg
2150100011730	PK1B	Proteinase K, 1 g

#### Storage:

- PK2 -20°C
- PK1 20°C

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Polymerase Chain Reaction (PCR) is an effective tool to synthesize defined sequences of DNA using thermostable polymerases. PCR exploits the inherent stability of these enzymes at high temperatures to amplify about a million copies of desired DNA fragment using two specific oligonucleotide primers that anneal to opposite strands flanking the target DNA sequence to be amplified. PCR is now widely used in cloning and sequencing DNA fragments, as a diagnostic tool and to generate labelled probes. To cater to these varied applications, Genei has a range of PCR enzymes, buffers, kits and optimized protocols.

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# Thermophilic Polymerase Selection Guide:

Routine PCR, RAPD RT-PCRColony PCR High through put PCR PCR based Diagnosis Multiplex PCR Note: Taq pol and its variant can be used in all the above-mentioned applications, choice is based on the end user requirements for yield, specificity etc., Hot Start Enzymes are preferred for Sensitive applications. Cene Cloning Long PCR Long RT-PCR Invitro Mutagenesis XT-PCR systems are	(amondo)
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Gene Cloning Long PC Long RT-PCR Invitro Mutagenesis XT-PCR systems are	•
Invitro Mutagenesis XT-PCR systems are	•
	•
• • preferred for Long PCR	•



BetterBest

To be supplemented with MAGIC Amplification Solution

# GeNei "

# Taq Polymerase

#### Description:

Taq DNA Polymerase is a 94 kDa enzyme known for its thermostability, making it ideal for DNA amplification. Its optimal activity temperature falls between 55°C and 75°C. What sets Taq apart is its absence of 3' to 5' exonuclease (proofreading) activity. However, it possesses a 5' to 3' exonuclease activity, allowing it to trim nucleotides from the 5' end of a DNA strand when necessary.

#### Unique Properties: >

- Amplification of DNA fragments of varied sizes ranging from about 100 bp to 3kb by PCR.
- The enzyme can be used in RAPD studies to detect polymorphism.
- Seed testing-GMO detection
- DNA fingerprinting-Forensics
- Diagnostics-DNA based.
- Nucleases free enzymes.

Molecular weight: 94 kDa monomer

**Storage conditions:** The Taq Polymerase should be stored at -20°C. Stable up to 3 year on recommended conditions.

Specification: The enzyme is supplied at concentrations of  $1U/\mu l$ ,  $3U/\mu l$ .

One unit is defined as the amount of enzyme required to convert 10 nmoles of total deoxyribonucleoside-triphosphates into acid precipitable DNA in 30 minutes at 72°C.

#### Taq DNA Polymerase Buffers:

Buffer	Concentration	10X Buffer Composition
Taq Buffer A	10X	Tris (pH9.0), KCl, 15 mM MgCl <sub>2</sub> , Gelatin
Taq Buffer B	10X	Tris (pH9.0), KCl, Gelatin
Taq Buffer E	10X	Tris (pH 9.0), KCl, 15mM MgCl <sub>2</sub> , TritonX-100
Taq Buffer F	10X	Tris (pH 9.0), KCl, TritonX-100

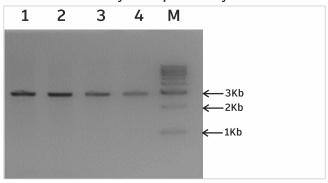
## Quality control assays:

**Absence of Nuclease Activity:** Taq DNA Polymerase when incubated for 16 hours with 1 g of DNA digest at 37° C and 72° C showed sharp unaltered pattern on 1% agarose gel.

Absence of Nickase Activity: Taq DNA Polymerase when incubated for 16 hours with 1 g of supercoiled plasmid DNA at 37°C and 72°C showed unaltered pattern without nicking on 1% agarose gel.

Thermo Stability: Taq DNA Polymerase was incubated at 94°C for 1 hour and subsequently checked for activity. >90% activity was retained as determined by primer extension assay.

#### Performance Assay of Taq DNA Polymerase



Amplification of 3.0 kb tPa gene using 100 ng of Human Genomic DNA as template with different amount of enzyme

Lane 1 - 2.5 units of enzyme

Lane 2 - 1.0 units of enzyme

Lane 3 - 0.5 units of enzyme

Lane 4 - 0.3 units of enzyme

Lane M - StepUp 1 Kb DNA ladder

Remarks: Specific amplicon with linear

decrease in the amount of  $\,$  amplicon observed from 2.5units to 0.3 units of enzyme.

## References:

 Laboratory Approaches in Molecular Pathology—The Polymerase Chain Reaction W.B. Coleman, G.J. Tsongalis, in Diagnostic Molecular Pathology, 2017

## Publications with MME5:

- Optimization of PCR reagents for amplification of microsatellites in oil palm M. Jayanthi\*, G. Sujatha and P.K. Mandal National Research Centre for Oil Palm Pedavegi, West Godavari District
- Development of tomato (solanum lycopersicon) lines with tolcv resistance gene and their authentication through molecular marker D.
   Datta, Aashish kumar, H.C. Prasanna, Sanjeev kumar, A.B. Rai and Mathura Rai Indian Institute Of Vegetable Research, P.O. Jakhini-Shahanshahpur, Varanasi.



Cat. No	PI No.	Taq DNA Polymerase (Supplied with 10X Buffer containing Gelatin and 15 mM MgCl <sub>2</sub> )
0601600031730	MME5L	Taq DNA Polymerase (3 U/µl) (Includes Enzyme: 1 vial;10X Taq Buffer A: 1 vial), 250Units
0601600051730	MME5J	Taq DNA Polymerase (3 U/µl) (Includes Enzyme: 1 vial;10X Taq Buffer A: 4 vials), 1000Units
0601600061730	MME5B	Taq DNA Polymerase, 2 x 2500U (3 U/μl) (Includes Enzyme: 2 vials;10X Taq Buffer A: 7 bottles x 3 ml), 5000Units
0602300051730	MME23L	Taq DNA Polymerase (5 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer A: 4 vials), 1000Units
0602300061730	MME23B	Taq DNA Polymerase, 2 x 2500U (5 U/µl) (Includes Enzyme: 2 vials; 10X Taq Buffer A: 7 bottles x 3 ml), 5000Units
0602400051730	MME24L	Taq DNA Polymerase (1 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer A: 4 vials), 1000Units
		Taq DNA Polymerase (Supplied with 10X Buffer containing Gelatin, separate vial of 25 mM MgCl₂)
0602500051730	MME25J	Taq DNA Polymerase (3 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer B: 4 vials; 25 mM MgCl <sub>2</sub> : 4 vials), 1000Units
602500061730	MME25B	Taq DNA Polymerase, 2 x 2500U(3 U/ $\mu$ l) (Includes Enzyme: 2 vials; 10X Taq Buffer B: 7 bottles x 3 ml; 25 mM MgCl <sub>2</sub> : 7 bottles x 3 ml), 5000Units
0602700051730	MME27L	Taq DNA Polymerase (5 U/μl) (Includes Enzyme: 1 vial; 10X Taq Buffer B: 4 vials; 25 mM MgCl <sub>2</sub> : 4 vials), 1000Units
0602700061730	MME27B	Taq DNA Polymerase, 2 x 2500U (5 U/ $\mu$ l) (Includes Enzyme: 2 vials; 10X Taq Buffer B: 7 bottles x 3 ml; 25 mM MgCl <sub>2</sub> : 7 bottles x 3 ml), 5000Units
0602800051730	MME28L	Taq DNA Polymerase (1 U/ $\mu$ l) (Includes Enzyme: 1 vial; 10X Taq Buffer B: 4 vials; 25 mM MgCl $_2$ : 4 vials), 1000Units
		Taq DNA Polymerase (Supplied with 10X Buffer containing TritonX-100 and 15 mM MgCl₂)
0602900051730	ММЕ29Ј	Taq DNA Polymerase (3 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer E: 4 vials), 1000Units
0603000051730	MME30L	Taq DNA Polymerase (5 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer E: 4 vials), 1000Units
0603100051730	MME31L	Taq DNA Polymerase (1 U/ $\mu$ l) (Includes Enzyme: 1 vial; 10X Taq Buffer E: 4 vials), 1000Units
		Taq DNA Polymerase (Supplied with 10X Buffer containing TritonX-100, separate vial of 25 mM MgCl <sub>2</sub> )
0603200051730	ММЕЗ2Ј	Taq DNA Polymerase (3 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer F: 4 vials; 25 mM MgCl2: 4 vials), 1000Units
0603300051730	MME33L	Taq DNA Polymerase (5 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer F: 4 vials; 25 mM MgCl2: 4 vials), 1000Units
0603400051730	MME34L	Taq DNA Polymerase (1 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer F: 4 vials; 25 mM MgCl2: 4 vials), 1000Units



#### **Buffers:**

Cat. No	PI No.	Product Description	
		Taq DNA Polymerase Buffers containing Gelatin	
0653100011730	METB1	Taq Buffer A (Tris with 15 mM MgCl <sub>2</sub> ),1ml	
0653200011730	METB2	Taq Buffer B (Tris without MgCl <sub>2</sub> ), 1ml	
		Taq DNA Polymerase Buffers containing TritonX-100	
0653300011730	METB13	Taq Buffer E (1 ml) (Tris with 15 mM MgCl <sub>2</sub> ),1ml	
0653400011730	METB14	Taq Buffer F (1 ml) (Tris without MgCl <sub>2</sub> ), 1ml	
0653500011730	METB5	MgCl <sub>2</sub> 25mM, 2ml.	

## **PCR Master Mix**

PCR master mix is a versatile and indispensable tool in molecular biology and genetics, enabling the amplification, detection, and analysis of DNA in numerous research applications. Its convenience and consistency make it a valuable resource in the laboratory.

The PCR Master Mix is a pre-prepared reagent with a 2X concentration, making it highly convenient for instant and efficient DNA amplification. This Master Mix includes Genei's Tag DNA Polymerase with deoxyribonucleotide triphosphates (dNTPs), and an optimized reaction buffer(1.5mMMagnesium Chloride),. This ready-to-use mixture ensures robust and dependable performance, making it an excellent choice for routine PCR (Polymerase Chain Reaction) applications. It simplifies the PCR process, allowing for quick and reliable results, and is a valuable tool in molecular biology and genetics research. It is supplied in 2X concentration to accommodate the addition of template primers, and (if necessary) addition of Magnesium chloride, additives like BSA, DMSO, glycerol, etc.,

# Key Highlights: >

- User-Friendly: Easy to use, simplifying laboratory procedures.
- Enhanced Consistency: Reduces experimental variabilities, ensuring reliable results.
- Contamination Prevention: Minimizes the risk of contamination for accurate outcomes.

- Reliable Performance: Provides consistent and dependable results.
- ◆ Time-Efficient: Saves time in the setup of reactions, streamlining laboratory workflows.

Storage: -20°C

#### Applications:

 DNA Amplification: PCR master mix is primarily used for DNA amplification, enabling the selective and exponential replication of specific DNA sequences. This application is fundamental to a wide range of downstream experiments.

#### References:

https://en.wikipedia.org/wiki/Master\_mix\_(PCR)

# Plant Direct GenAmp PCR Kit

## Description:

The Plant Direct GenAmp PCR Kit is fast, easy to perform and consequently ideal for large sample numbers. In addition, only a very small sample (0.5 cm leaf punch, for example) is enough for the assay. The protocol involves direct PCR amplification from the plant material without a DNA extraction step. The leaf material is crushed in one buffer, followed by dilution and incubation in a second buffer. The Plant Direct GenAmp PCR Kit represents a considerable simplification of present approaches by eliminating DNA isolation prior to PCR amplification.

#### Unique Features: >

- Excludes the need for DNA extraction.
- Fast and simple protocol requiring minimal amount of sample.
- Ideal for large number of samples.
- Tested with a wide variety of plant species. ( Leaf samples of plants tested with Plant Direct GenAmp PCR Kit) 1. Tomato 2. Banana Citrus 3. Coriander 4. Spinach 5. Chilly 6. Catharanthus 7. Bhindi/ Ladies finger/Okra, 8. Cauliflower 9. Sorghum 10. Pumpkin 11. Arabidopsis 12. Jatropa 13. Ocimum 14. Piper Longum 15. Rice 16. Sugarcane 17. Rubber 18. Potato 19. Maize 20. Mulberry 21. Onion 22. Papaya 23. Arabidopsis 24. Tobacco 25. Mustard 26. Wheat.

#### Key Contents:

- I-Buffer
- Forward Primer (Control Primer Set (A)
- Reverse Primer (Control Primer Set (A)
- 10X Buffer for HotStart Gen Amp DNA Polymerase
- dNTPs Mix (2.5 mM each)
- HotStart Gen Amp DNA Polymerase
- PCR Grade Water
- G-Buffer
- Tissue Grinders

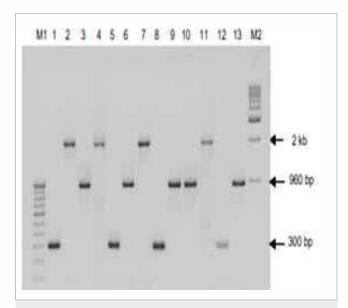


Fig 1: Amplification of 300bp, 960 bp and 2Kb fragments/amplicons from different plant leaf samples using Plant Direct GenAmp PCR Kit.

#### M1 - StepUp™100 bp DNA ladder

**Lane 1** - Citrus leaf sample amplified with Primer Set A (300bp)

Lane 2 - Mulberry leaf sample amplified with Primer Set C (2Kb)

Lane 3 - Catharanthus leaf sample amplified with primer Set B (960bp)

Lane 4 - Rice leaf sample amplified with primer Set C (2 Kb)

Lane 5 - Catharanthus leaf sample amplified with Primer Set A (300bp)

**Lane 6** - Banana leaf sample amplified with Primer Set B (960bp)

Lane 7 - Citrus leaf sample amplified with Primer Set C (2Kb)

Lane 8 - Banana leaf sample amplified with Primer Set A (300bp)

**Lane 9** - Mulberry leaf sample amplified with Primer Set B (960 bp)

Lane 10 - Rice leaf sample amplified with Primer Set B (960 bp)

Lane 11 - Catharanthus leaf sample amplified with Primer Set C (2 Kb) Lane 12 - Rice leaf sample amplified with Primer Set A (300 bp)

Lane 13 - Citrus leaf sample amplified with Primer Set B (960 bp)

#### M2 - StepUp™1 Kb DNA ladder

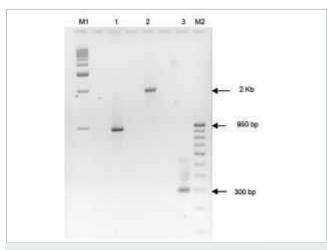


Fig 2: Amplification of 300bp, 960 bp and 2Kb fragments from Arabidopsis leaf sample using Plant Direct GenAmp PCR Kit.

#### M1 - StepUp™ 1 Kb DNA Ladder

Lane 1 - Arabidopsis leaf sample amplified with primer set B (1 Kb)

Lane 2 - Arabidopsis leaf sample amplified with primer set C (2 Kb)

Lane 3 - Arabidopsis leaf sample amplified with primer set A (300 bp)

- StepUp™100 bp DNA Ladder

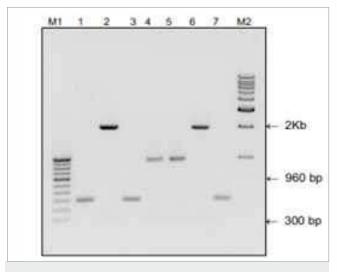


Fig 3: Amplification of 300bp, 960 bp and 2Kb fragments from different seed samples using Plant Direct GenAmp PCR Kit

#### M1 - StepUpTM100 bp DNA ladder

Lane 1 - Wheat seed sample amplified with Primer Set A (300bp)

Lane 2 - Mustard seed sample amplified with Primer Set C (2Kb)

Lane 3 - Tobacco seed sample amplified with primer Set A (300bp)

Lane 4 - Tobacco seed sample amplified with primer Set B (960 bp)

Lane 5 - Tomato seed amplified with Primer Set B (960bp)



Lane 6 - Tomato seed amplified with Primer Set C (2 Kb)
 Lane 7 - Arabidopsis seed amplified with Primer Set A (300 bp)
 M2 - StepUpTM 1 Kb DNA Ladder

#### Key Contents:

 This kit is useful in plant studies and downstream applications like High throughput plant genomic PCR, Phylogenetic or population-based plant DNA barcoding, plant seed fidelity testing etc. It is also suitable for DNA amplification across a broad range of plant species

#### References:

- Doyle, J. J. and J. L. Doyle (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.
- Taberlet, P. L., P. Gielly, G. Patou, and J. Bouvet (1991). Universal primers for the amplification of three noncoding regions of chloroplast DNA. Plant Molecular Biology 17: 1105–1109.
- Aljanabi, S. M., and I. Martinez. (1997). Universal and rapid salt extraction of high quality DNA for PCR-based techniques. Nucleic Acids Research 25: 4692 – 4693.

#### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
0681300011730	KT234M	Plant Direct GenAmp PCR Kit, 50 reactions
0681300021730	KT234L	Plant Direct GenAmp PCR Kit,250 reactions

# GeNei™ DNA Amplification Reagent Kit (with Marker)

# Description: >

The Polymerase Chain Reaction Invented in 1983 by Kary Mullis or PCR is a reliable and routinely used tool in Molecular Biology and Biotechnology. The purpose of PCR is to rapidly amplify and make a large number of copies of any gene starting from a very small number. Kary Mullis was awarded the Nobel Prize in Chemistry for his work in 1993.

The reaction is very simple and requires no more than a test tube, a few simple reagents and a source of heat. The reaction mixture comprises of the following:

- A target or template dsDNA which is the DNA that is to be amplified I Two short and specific primers which are responsible for initiation of amplification called forward and reverse primers. They contain sequences complementary to the target DNA and are single stranded
- A thermostable DNA polymerase which brings about the polymerization of the dNTPs.
- dNTPs or nucleotides which get added to form the new strand - the DNA building blocks
- Buffers including divalent and monovalent cations to support the reaction

#### Key Contents:

- Tag DNA Polymerase
- dNTPs
- 10X Assay Buffer with 15mM MgCl<sub>2</sub>
- 10X Assay Buffer without MgCl<sub>2</sub>
- 25mM MgCl<sub>2</sub>
- Control DNA and Primers
- DNA Marker.
- Instruction Manual.

#### Storage: -20°C

## References:

- Saiki R.K et.al. (1985) Science 230, 1350-1354.
- Mullis K.B et.al. (1987) Methods Enzymol. 155, 335-350
- Saiki R.K et.al. (1988) Science 239, 487-491.
- PCR Technology, H. Erlich Ed., Stockton press, New York, 1989.
- PCR Topics, A. Rolfs, H.C. Schumacher, P. Marx Eds. Springer-Verlag, New York, 1991.
- PCR Protocols, Current methods and applications Ed. Bruce A. White, Volume 15, Humana press, Totowa, New Jersey,1993.

Cat. No	PI No.	<b>Product Description</b>
0660300051730	KT03	GeNei™ DNA Amplification Reagent Kit (with Marker) ,100 reactions
0660500011730	KT03I	GeNei™ DNA Amplification Kit (with Marker), 50 reactions
0660400051730	КТОЗС	GeNei™ DNA Amplification Core Kit (with Marker), 100 reactions

# GenAmp Direct PCR Kit

(for Mammalian Cell lines, Bacteria and Yeast)

#### Description: >

The Direct PCR Kit for Mammalian Cell lines, Bacteria and Yeast enables DNA amplification directly from mammalian cell lines, bacteria and yeast samples without any prior DNA extraction. The protocol is fast and easy, without the need for steps like phenol extraction, DNA precipitation and column purification. The method involves grinding and incubation of various cell pellets in lysis buffer for 10 minutes at 95° C.Cool at RT add lysis buffer II and spin.After centrifugation, 1-2  $\mu l$  of the supernatant can be used directly for PCR amplification. The GenAmp Direct PCR Kit serves as a simple, quick and inexpensive method for amplification of genomic DNA from various samples like bacteria, yeast and mammalian cell lines.

## Unique Features: >

- Direct amplification of genomic DNA from various sources like bacteria, yeast and mammalian cell lines.
- Simple buffer system without the need of Proteinase K treatment.
- Consistent highly reproducible with low turnaround time.
- Fast and easy protocol Eliminates need for a separate DNA extraction step prior to PCR and enables rapid extraction of DNA from cells.

# Applications:

- Genomic DNA amplification from various mammalian cell lines, bacteria and yeast.
- Molecular biology and PCR applications

#### Key Contents: >

- Red Dye PCR Master Mix (2X)
- Lysis Buffer I
- Lysis Buffer II



**Fig1:** Analysis of PCR products of 2 different sizes from 3 strains of Bacteria using GenAmp Direct PCR Kit (for Mammalian Cell lines, Bacteria and Yeast) on Agarose gel.

#### M1: StepUp™ 100 bp DNA ladder

- **Lane 1 -** Staphylococcus Aureus amplified with primer set giving an amplicon size of 411 bp
- Lane 2  $\,$  DH5  $\!\alpha$  amplified with primer set giving an amplicon size of 411 bp
- **Lane 3 -** Bacillus Subtilis amplified with primer set giving an amplicon size of 411 bp
- Lane 4 Negative PCR control
- **Lane 5 -** Staphylococcus Aureus amplified with primer set giving an amplicon size of 1.5 Kb
- Lane 6 DH5 $\alpha$  amplified with primer set giving an amplicon size of 1.5 Kb
- Lane 7 Bacillus Subtilis amplified with primer set giving an amplicon size of 1.5 Kb

#### M2: StepUp™ 1 Kb DNA ladder

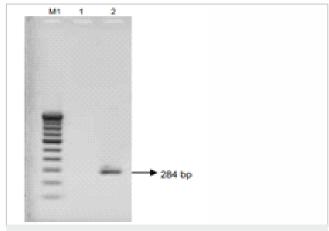
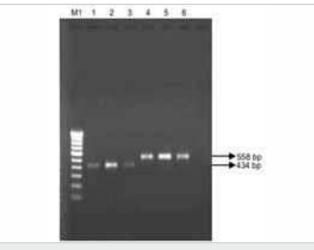


Fig 2: Analysis of PCR product from Pichia Pastoris strain of yeast using GenAmp Direct PCR Kit (for Mammalian Cell lines, Bacteria and Yeast) on 1.5% Agarose gel.

- M1 StepUp™ 100 bp DNA ladder
- Lane 1 Negative PCR control
- Lane 2 Pichia Pastoris amplified with primer set giving an amplicon size of 284 bp

# GeNei<sup>®</sup>



**Fig 3:** Analysis of PCR products of 2 different sizes from 3 mammalian cell lines using GenAmp Direct PCR Kit (for Mammalian Cell lines, Bacteria and Yeast) on 1.5% Agarose gel.

#### M1: StepUp™ 100 bp DNA ladder

- **Lane1** Jurkat cell pellet amplified with primer set giving an amplicon size of 434 bp
- Lane 2 MCF-7 cell pellet amplified with primer set giving an amplicon size of 434 bp
- **Lane 3** HEK 293 cell pellet amplified with primer set giving an amplicon size of 434 bp
- $\textbf{Lane 4}\,$   $\,$  Jurkat cell pellet amplified with primer set giving an amplicon size of 558 bp
- $\textbf{Lane 5}\,$  MCF-7 cell pellet amplified with primer set giving an amplicon size of 558 bp
- **Lane 6 -** HEK 293 cell pellet amplified with primer set giving an amplicon size of  $558\,\mathrm{bp}$

#### References:

- Rudbeck, L. and J. Dissing. (1998). Rapid, simple alkaline extraction of human genomic DNA from whole blood, buccal epithelial cells, semen and forensic stains for PCR BioTechniques 25: 588-592
- Von Ashen, N., M. Oellerich, and E. Schutz. (2000). Use of two reporter dyes without interference in single-tube rapid-cycle PCR: alpha (1)antitrypsin genotyping by multiplex real-time fluorescence PCR with the Light Cycler Clin. Chem. 46:156-161.
- Planelles, D., F. Llopis, N. Puig and J.A. Montoro. (1996). A new, fast andz simple DNA extraction method for HLA and VNTR genotyping by PCR amplification. J. Clin. Lab. Anal. 10: 125-128
- Saiki R.K et.al. (1985) Science 230, 1350-1354.
- Mullis K.B et.al. (1987) Methods Enzymol. 155, 335-350
- Saiki R.K et.al. (1988) Science 239, 487-491.
- PCR Technology, H. Erlich Ed., Stockton press, New York, 1989.
- PCR Topics, A. Rolfs, H.C. Schumacher, P. Marx Eds. Springer-Verlag, New York, 1991.
- PCR Protocols, Current methods and applications Ed. Bruce A. White, Volume 15, Humana press, Totowa, New Jersey, 1993.

Cat. No	PI No.	<b>Product Description</b>
2125300021730	KT253	GeNei™ Direct PCR Kit ( for Mammalian Cell lines, Bacteria & Yeast), 50 reactions

# Mouse Tissue Direct GenAmp PCR Kit

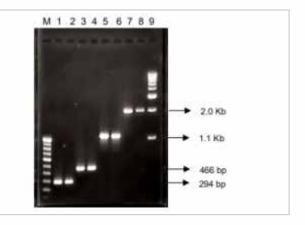
The Mouse Tissue Direct PCR Kit enables DNA amplification directly from mouse tissues like liver, kidney, spleen, heart, lungs, ear and tail without the need of time-consuming conventional DNA extraction. The protocol is fast and easy, without the need for steps like tissue homogenisation, phenol extraction, DNA precipitation and column purification. In addition, it does not require overnight incubation and Proteinase K treatment. The method involves incubation of 1-2 mm of mouse tissue in lysis buffer for 10 minutes followed by dilution with the second buffer (without any further incubation). 1-2 µl of this lysate can be used directly for PCR amplification. The Mouse tissue Direct PCR Kit serves as a simple, quick and inexpensive method for amplification of genomic DNA from various mouse tissues.

## Unique Features: >

- Direct amplification of genomic DNA from various mouse tissues.
- Simple buffer system without the need of Proteinase K treatment.
- Consistent highly reproducible with low turnaround time.
- Fast and easy protocol Eliminates need for a separate DNA extraction step prior to PCR and enables rapid extraction of DNA from tissues.
- Forward Primer (Control Primer Set A)

# Key Contents:

- Reverse Primer (Control Primer Set A)
- HotStart Master Mix (2X)
- Lysis Buffer I
- Lysis Buffer II
- Nuclease free water
- Tissue Grinder



**Fig 1:** Analysis of 294 bp, 466 bp, 1.1 Kb and 2 Kb amplicon from mouse tail and ear tissues using Mouse Tissue Direct GenAmp PCR Kit, on 1.5% Agarose gel.

#### M:StepUp™ 100 bp DNA ladder

**Lane 1** - 294 bp amplicon from Mouse tail tissue

Lane 2 - 294 bp amplicon from Mouse ear tissue

Lane 3 - 466 bp amplicon from Mouse tail tissue

**Lane 4** - 466 bp amplicon from Mouse ear tissue

Lane 5 - 1.1 Kb amplicon from Mouse tail tissue
Lane 6 - 1.1 Kb amplicon from Mouse ear tissue

Lane 7 - 2 Kb amplicon from Mouse tail tissue

Lane 8 - 2 Kb amplicon from Mouse ear tissue

#### Applications: >

- Mouse genotyping.
- Genomic DNA amplification from various mouse tissues

#### References:

- Harald, L., Andreas, H., N. Rieger, R. D. Wanke and E. Wolf. (1998).
   Identification of transgenic mice by direct PCR analysis of lysates of epithelial cells obtained from the inner surface of the rectum.
   Transgenic Research 7: 131-134
- Drews, R., W. N. Drohan and H. Lubon. (1994). Transgene detection in mouse tail digests. BioTechniques 17: 866-867. 3. Chen, S., and G. A. Evans. (1990). A simple screening method for transgenic mice using the polymerase chain reaction. BioTechniques 8: 32-33.

Cat. No	PI No.	<b>Product Description</b>
2125400021730	KT254	Mouse Tissue Direct GenAmp PCR Kit, 50 reactions



# Red Taq polymerase

Red Taq DNA Polymerase is a blend of Taq DNA Polymerase and an inert red dye. This dye enables quick visual confirmation of enzyme addition and reaction mixing. After amplification, the product can be directly loaded on to agarose gel without the addition of gel loading dye. The mobility of the red dye is slightly ahead of bromophenol blue dye.

Unit Definition: One unit is defined as the amount of enzyme required to convert 10 nmoles of total deoxyribonucleoside-triphosphates into acid precipitable DNA in 30 minutes at 72° C.

Reagents Supplied with Enzyme: 10X Assay Buffer (Taq Buffer A). 1X Assay Buffer: 10 mM Tris-HCl (pH 9.0 at 25°C), 1.5 mM MgCl<sub>2</sub>,50 mM KCl and 0.01% gelatin.

Storage: Store enzyme and assay buffer at -20°C.

**Note:** On observation of any kind of precipitate in Taq Buffer A warm the buffer at 35-40°C till precipitate dissolves

#### Quality control assays: >

- Absence of Nuclease Activity: Red Taq DNA Polymerase when incubated for 16 hours with 1 g of DNA digest at 37°C and 72°C showed sharp unaltered pattern on 1% agarose gel.
- Absence of Nickase Activity: Red Taq DNA
   Polymerase when incubated for 16 hours with 1 g
   of supercoiled plasmid DNA at 37°C and 72°C
   showed unaltered pattern without nicking on 1%
   agarose gel.

#### Performance Test:

- Red Taq DNA Polymerase was used in RAPD of rice DNA with a 10 mer primer. Expected band pattern was observed on 1.8% agarose gel.
- Red Taq DNA Polymerase was routinely used for generating specific amplified products of varying sizes from 100 bp to 3 kb with different template sources.

#### References:

 Laboratory Approaches in Molecular PathologyThe Polymerase Chain Reaction W.B. Coleman, G.J. Tsongalis, in Diagnostic Molecular Pathology, 2017

Cat. No	PI No.	<b>Product Description</b>		
GeNei™ Red Taq DNA Polymerase (Supplied with 10X Buffer containing Gelatin and 15 mM MgCl₂)				
0603500051730	MME35J	GeNei <sup>™</sup> Red Taq DNA Polymerase (1 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer A: 4 vials), 1000Units		
GeNei <sup>TM</sup> Red Taq DNA Polymerase (Supplied with 10X Buffer containing Gelatin, separate vial of 25 mM MgCl <sub>2</sub> )				
0603600051730	ММЕЗ6Ј	GeNei™ Red Taq DNA Polymerase (1 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer B: 4 vials; 25 mM MgCl <sub>2</sub> : 4 vials), 1000Units		
GeNei™ Red Taq DNA Polymerase (Supplied with 10X Buffer containing TritonX-100 and 15 mM MgCl <sub>2</sub> )				
0603700051730	ММЕЗ7Ј	GeNei™ Red Taq DNA Polymerase (1 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer E: 4 vials), 1000Units		

# GeNei™ Red Dye PCR Master Mix (2X)

### Description:

The Red dye Master Mix consists of a unique inert red dye in addition to all basic components necessary to do PCR: Taq DNA Polymerase, dNTPs, reaction buffer (with 1.5 mM Magnesium chloride) at optimum concentrations. The mobility of the red dye is slightly ahead of bromophenol blue dye. The Genei Red dye PCR Master Mix is supplied at 2X concentration to accommodate the addition of template, primers and (if necessary) addition of magnesium chloride, additives like BSA, DMSO, glycerol, etc.

What sets this PCR Master Mix apart is its unique formulation, allowing for direct loading of the amplified product onto an agarose gel without the necessity of additional gel loading dye. This streamlined approach not only saves time but also enhances the overall workflow efficiency.

Whether utilized for PCR generated fragments or genomic DNA amplification through techniques such as PCR or restriction digestion, this Ready To Use Red Dye PCR Master Mix guarantees robust and dependable performance. Its versatility, coupled with the elimination of additional steps in gel loading, makes it an invaluable tool for molecular biology laboratories seeking efficiency without compromising on quality

#### Note

- Aliquot 2X Genei Red Dye Master Mix into single use tubes to prevent multiple freeze thaw cycles and to minimize contamination.
- Use PCR Master Mix supplied at 1X concentration
- Mix can be used to perform PCR reaction of volumes greater than 25  $\mu$ l.
- Lysis Buffer I
- Lysis Buffer II
- Nuclease free water
- Tissue Grinder

### Direction to use:

- Thaw the master mix, gently mix and aliquot 12.5 µl into PCR tubes.
- Add template, primers and (If required, add additional magnesium chloride, additives like DMSO, glycerol etc.,) and make up the volume to 25 µl
- Start the thermal cycling.
- Load the samples directly on to agarose gel.

### Quality control assays: >

- Absence of Nuclease Contamination: Individual components of the mix have been extensively tested for the absence of any contaminating nucleases.
- **Performance Test:** Genei Red Dye master mix is tested for amplification of 0.8 kb and 2.1 kb fragments using respective template and specific primer combinations.

### Key Highlights:

- User-Friendly: Simplifying your laboratory workflow, this product is designed for ease of use, catering to both novice and experienced researchers.
- **Direct Gel Loading:** The convenience of loading the product directly onto the gel streamlines the experimental process, saving time and effort in sample preparation.
- Reduced Experimental Variabilities: Ensuring reliability in your results, this product minimizes experimental variabilities, contributing to the consistency of your data.
- Minimal Contamination Risk: With a focus on maintaining the integrity of your samples, the design of this product minimizes the risk of contamination, enhancing the reliability of your experiments.
- Consistent Results: The product is engineered to deliver consistent and reproducible results, providing a reliable foundation for your research outcomes.
- Time-Efficient: By saving reaction setup time, this



product contributes to the efficiency of your experimental pipeline, allowing you to allocate your time and resources more effectively.

### Related Product-KT78:

This kit consists of all the reagents required to perform 100 standard PCR reactions.

### Materials Provided:

- 5 vials of Red Dye PCR Master Mix(2X)
- Sterile water
- ◆ StepUp<sup>™</sup> 100bp DNA ladder (Ready to use)

Storage: -20°C

### Quality control assays:

- Absence of Nuclease Activity: Red Taq DNA Polymerase when incubated for 16 hours with 1 g of DNA digest at 37°C and 72°C showed sharp unaltered pattern on 1% agarose gel.
- Absence of Nickase Activity: Red Taq DNA Polymerase when incubated for 16 hours with 1 g of supercoiled plasmid DNA at 37°C and 72°C showed unaltered pattern without nicking on 1% agarose gel.

### Applications:

- Routine PCR: Standard PCR for amplifying DNA fragments for various applications, such as cloning, sequencing, or genotyping.
- Endpoint PCR: For basic DNA amplification where the final PCR product is visualized by gel electrophoresis or other detection methods.
- Colony PCR: Identification of bacterial colonies carrying the desired plasmid or DNA fragment.
- Screening PCR: High-throughput screening of samples for the presence or absence of specific DNA sequences.
- Genotyping: Analysis of genetic variations, such as single nucleotide polymorphisms (SNPs) or insertions/deletions, in a population.
- RT-PCR (Reverse Transcription PCR): For the amplification of complementary DNA (cDNA) from RNA templates, commonly used in gene expression studies.

- Multiplex PCR: Simultaneous amplification of multiple target DNA sequences in a single reaction.
- Forensic PCR: Amplification of DNA for forensic analysis, such as DNA profiling and paternity testing.

### **Ordering Information**

Cat. No	PI No.	Product Description
0602100011730	MME21S	GeNei™ Red Dye PCR Master Mix (2X), 100 reactions
0602100031730	MME21	GeNei™ Red Dye PCR Master Mix (2X), 5X100 reactions
0602100051730	MME21L	GeNei™ Red Dye PCR Master Mix (2X), 2000 reactions
0667800041730	KT78	GeNei™ Red Dye PCR Master Mix Kit (2X) (with 100 bp DNA Ladder). 5X20 reactions

# GeNei™ HotStart Taq DNA Polymerase

### Description:

HotStart Taq DNA Polymerase is an antibody based HotStart Taq DNA Polymerase, that possesses 5' 3' polymerase activity and 5' 3' exonuclease activity.

Unit Definition: One unit is defined as the amount of enzyme required to convert 10 nmoles of total deoxyribonucleoside-triphosphates into acid precipitable DNA in 30 minutes at 72°C.

**Storage Buffer:** Taq DNA Polymerase is supplied in Tris-HCl (pH 8.0), KCl, EDTA, DTT, Tween -20, Igepal and Glycerol.

Reagents Supplied with Enzyme: 10X Assay Buffer (Taq Buffer A). 1X Assay Buffer: Tris-HCl (pH 9.0 at 25°C), 1.5 mM MqCl2, KCl and gelatin.

# **GeNei**

### Quality control assays: >

- Absence of Nuclease Activity: 6 units of HotStart Taq DNA Polymerase when incubated for 16 hours with 1 g of DNA digest at 37°C and 72°C showed sharp unaltered pattern on 1% agarose gel.
- Absence of Nickase Activity: 9 units of HotStart Taq DNA Polymerase when incubated for 16 hours with 1 g of supercoiled plasmid DNA at 37°C and 72°C showed unaltered pattern without nicking on 1% agarose gel.
- Inhibition/Reactivation assay: Inhibition of activity in HotStart Taq DNA Polymerase was evaluated in DNA polymerization activity assay that measured the percent of enzyme inhibition at 60°C for 1 hour. More than 95% of enzyme activity was found to be inhibited. On reactivation at 95°C for 5 minutes, complete activity was restored. Polymerization activity of Taq DNA Polymerase was the referral control used.

### Performance Test: >

- Hotstart Taq DNA Polymerase is tested extensively for its reproducible performance in critical PCR amplifications and in RT-PCR.
- Enzyme is tested for amplification of 131 bp fragment of TNF gene and 400 bp fragment of globin gene from human genomic DNA using nonoptimal primers.
- Enzyme is tested for detecting different subtypes of Human Papilloma virus (HPV) using consensus primers

#### Storage: Store enzyme and assay buffer at -20°C.



Amplification of 250 bp fragment with varied sample dilutions using consensus primers  $% \left( 1\right) =\left( 1\right) \left( 1\right) \left($ 

Lane 1 & 14 - StepUp™ 100 bp DNA Ladder

Lane 2-7 - Amplification with Taq DNA Polymerase (10-1 to 10-6 dilutions)

Lane 8-13 - Amplification with HotStart Taq DNA Polymerase (10-1 to 10-6 dilutions)

### References:

- Sharkey DJ, Scalice ER, Christy KG, Atwood SM, Daiss JL (May 1994).
   "Antibodies as thermolabile switches: high temperature triggering for the polymerase chain reaction"
- Kaboev, O. K.; Luchkina, L. A.; Tret'iakov, A. N.; Bahrmand, A. R. (2000-11-01). "PCR hot start using primers with the structure of molecular beacons (hairpin-like structure)". Nucleic Acids Research.
- Hotstart Taq DNA Polymerase is tested extensively for its reproducible performance in critical PCR amplifications and in RT-PCR.
- Enzyme is tested for amplification of 131 bp fragment of TNF gene and 400 bp fragment of -globin gene from human genomic DNA using nonoptimal primers.
- Enzyme is tested for detecting different subtypes of Human Papilloma virus (HPV) using consensus primers

### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>	
GeNei™ HotStart Taq DNA Polymerase (Supplied with 10X Buffer containing Gelatin and 15 mM MgCl <sub>2</sub> )			
0602000031730	MME20L	GeNei™ HotStart Taq DNA Polymerase (3 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer A: 1 vial), 250Units	
0602000051730	ММЕ2ОЈ	GeNei™ HotStart Taq DNA Polymerase (3 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer A: 4 vials), 1000Units	
(Supplied wi	th 10X Buffe	nq DNA Polymerase r containing Gelatin, 5 mM MgCl <sub>2</sub> )	
0604000051730	ММЕ4ОЈ	GeNei™ HotStart Taq DNA Polymerase (3 U/µl) (Includes Enzyme: 1 vial; 10X Taq Buffer B: 4 vials; 25 mM MgCl2: 4 vials), 1000Units	

# GeNei TM

# GeNei™ HotStart PCR Master Mix (2X)

### Description:

HotStart PCR Master Mix is a premixed, ready-to-use solution, containing Hot start Taq DNA Polymerase, dNTPs, Standard Taq Reaction Buffer, and stabilizers. It is supplied in 2X concentration to accommodate the addition of template, primers and if necessary, addition of Magnesium chloride, additives like BSA, DMSO, glycerol, etc.

### Storage:

- At 4°C for immediate use.
- At -20°C for long term use

**Note:** Aliquote 2X HotStart PCR Master Mix into single use tubes to prevent multiple freeze thaw cycles and to minimize contamination.

### Features:

- Improves PCR specificity
- Saves valuable time
- Reduces the number of reagent handling steps
- Avoids chances of costly errors and improves reproducibility.
- Allows for room temperature reaction assembly
- Low contamination risk

### Kit Contents:

- I-Buffer
- Forward Primer (Control Primer Set (A)
- Reverse Primer (Control Primer Set (A)
- 10X Buffer for HotStart Gen Amp DNA Polymerase
- dNTPs Mix (2.5 mM each)
- HotStart Gen Amp DNA Polymerase
- PCR Grade Water
- G-Buffer
- Tissue Grinders

### Quality control assays: >

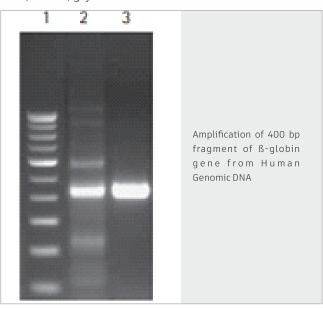
 Absence of Nuclease Contamination: Individual components of the mix have been extensively tested for the absence of any contaminating nucleases.  Performance Test: HotStart PCR Master Mix is tested extensively for its reproducible performance in critical PCR amplifications and in RT-PCR. Mix is tested for amplification of 131bp fragment of TNFgene and 400bp of -Globin gene from human genomic DNA using non-optimal primers. Mix is tested for detecting different subtypes of human Papilloma virus (HPV) using consensus primers.

#### Related Product: MME44

### Description:

The GeNei™ HotStart Blue Dye PCR Master Mix is a premixed, ready to use solution containing Hot Start Taq DNA Polymerase, dNTPs, standard reaction buffer, stabilizers and easy to track blue loading dye. Inclusion of tracking dye allows the direct loading & analysis of PCR products by Agarose gel electrophoresis following PCR amplification. The dye migrates at 5kb.

The GeNei<sup>™</sup> HotStart Blue Dye PCR Master Mix is supplied in 2X concentration to accommodate the addition of Magnesium chloride, additives like BSA,DMSO, glyceroletc.



Lane 1 - StepUp™ 100 bp DNA Ladder

Lane 2 - Amplification with PCR master mix Taq (400 bp)

Lane 3 - Amplification with HotStart PCR master mix (400 bp)

#### Storage:

At  $4^{\circ}$ C for immediate use. At  $-20^{\circ}$ C for long term use



### Applications: >

- High-specificity PCR amplification
- High-sensitivity PCR amplification
- TA-vector cloning.
- Amplification prior to in vitro transcription

### References:

- Sharkey DJ, Scalice ER, Christy KG, Atwood SM, Daiss JL (May 1994).
   "Antibodies as thermolabile switches: high temperature triggering for the polymerase chain reaction"
- Kaboev, O. K.; Luchkina, L. A.; Tret'iakov, A. N.; Bahrmand, A. R. (2000-11-01). "PCR hot start using primers with the structure of molecular beacons (hairpin-like structure)". Nucleic Acids Research.

### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
0604200011730	MME42S	GeNei™ HotStart PCR Master Mix (2X), 1 X 50 reactions
0604200031730	MME42L	GeNei™ HotStart PCR Master Mix (2X), 5 X 50 reactions
0604400021730	MME44	GeNei™ HotStart Blue Dye PCR mastermix (2X) 1X100 reactions

## Effi-Taq™ DNA Polymerase

### Description:

Effi-Taq™ DNA Polymerase a modified form of Taq DNA Polymerase is supplied in an inactive state that has no polymerase activity at ambient temperature. This prevents extension of non-specifically annealed primers and primer dimers formed at low temperature during PCR setup and initial PCR cycle. Effi-Taq™ DNA Polymerase is activated by a 20-minute incubation at 95°C, which can be incorporated into any existing thermal-cycler program. Every lot of Effi-Taq™ DNA Polymerase is subjected to a comprehensive range of quality control tests, including a stringent PCR specificity and reproducibility assay in which low-copy targets are amplified.

### Unique Features: >

- Higher Sensitivity
- Higher Specificity
- Convenient Room Temperature PCR Setup
- Minimal Optimization-saving time and money
- Generates PCR Products with 3'dA overhangs

### Unit Definition:

One unit is defined as the amount of enzyme required to convert 10 nmoles of total deoxyribonucleoside-triphosphates into acid precipitable DNA in 30 minutes at 72°C.

### Storage Buffer:

Taq DNA Polymerase is supplied in 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween -20, 0.5 % Igepal and 50% Glycerol & Stabilizers Reagents Supplied with Enzyme: 1vial each of 10X Assay Buffer containing 15 mM MgCl2, 25mM Mgcl2 & MAGIC Amplification solution.

### 1X Assay Buffer:

10 mM Tris-HCl (pH 9.0 at 25°C), 1.5 mM MgCl2, 50 mM KCl and 0.1% TritonX-100

### Quality control assays: >

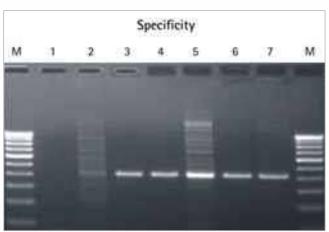
- Absence of Nuclease Activity: 10 units of Effi-Taq DNA Polymerase when incubated for 16 hours with 1 g of DNA digest at 37°C and 72°C showed sharp unaltered pattern on 1% agarose gel.
- Absence of Nickase Activity: 10 units of Effi-Taq DNA Polymerase when incubated for 16 hours with 1 g of supercoiled plasmid DNA at 37°C and 72°C showed unaltered pattern without nicking on 1% agarose gel.
- Inhibition/Reactivation assay: Inhibition of activity in Effi-Taq DNA Polymerase was evaluated in DNA polymerization activity assay that measured the percent of enzyme inhibition at 60°C for 1 hour. More than 95% of enzyme activity was found to be inhibited. On reactivation at 95°C for 20 minutes, activity was restored.



Polymerization activity of Taq DNA Polymerase was the referral control used.

### Performance Test:

- Assay for Sensitivity: Amplification of less than 10 copies of 201bp Male specific Target (SRY gene) in a high background of female DNA
- Assay for Specificity: A 400bp region of the human Beta globin was amplified using non-optimized primers.
- Assay for GC rich Templates: Amplification of an 80-85% GC-rich region of the human FMRI gene



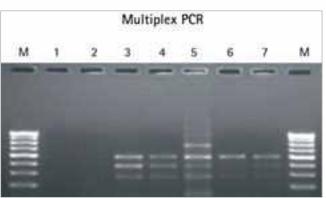
Amplification of 400bp region of the human ß-globin gene using non-optimized Primers

**Lane M** - SteUp<sup>™</sup> 100 bp DNA Ladder Lane 1 : Reagent Control

Lane 2 - Taq DNA Polymerase

**Lane 3** - Effi-Taq™ DNA Polymerase

Lane 4-7 - Competitor



Amplification of 5 different loci of Cotton Genomic DNA

**Lane M** - StepUp™ 100 bp DNA Ladder

Lane 1 - Reagent Control

Lane 2 - Tag DNA Polymerase

**Lane 3** - Effi-Taq™DNA Polymerase

Lane 4-7 - Competitor

### References:

- DNA polymerases as useful reagents for biotechnology the history of developmental research in the field". Frontiers in Microbiology. Ishino S, Ishino Y (2014).
- Domain exchange: chimeras of Thermus aquaticus DNA polymerase, Escherichia coli DNA polymerase I and Thermotoga neapolitana DNA polymerase"- Villbrandt B, Sobek H, Frey B, Schomburg D (September 2000).

 Improvement of the 3'-5' exonuclease activity of Taq DNA polymerase by protein engineering in the active site". Molecules and Cells.- Park Y, Choi H, Lee DS, Kim Y (June 1997).

### **Ordering Information:**

Cat. No PI No. Product Description

0604300031730 MME43L Effi-Taq™ DNA
Polymerase, 5
Units/µl, 250Units

### **PR Polymerase**

### Description: >

PR Polymerase, utilized in PCR reactions demanding high-fidelity synthesis, is a thermostable enzyme recognized for its robust Proof-Reading activity. The proofreading mechanism, facilitated by the 3'-5' exonuclease, allows the enzyme to correct misincorporated nucleotides, thereby minimizing errors during polymerization. This polymerase is well-suited for achieving high-fidelity amplifications, especially in short stretches of target DNA (< 1.5 kb). Its distinctive properties make it an ideal choice for applications where precision and accuracy are paramount.

PR Polymerase stands out as the enzyme of choice for high-fidelity DNA synthesis. It is particularly well-suited for high-fidelity PCR amplification of DNA fragments intended for applications such as gene cloning, site-directed mutagenesis, and various other genetic manipulations.

The enzyme's robust proofreading activity and thermostability make it a reliable tool for achieving accurate and precise amplification, crucial for downstream molecular biology applications. PR Polymerase is recommended for use in PCR and primer extension reaction that require high fidelity.

**Unique feature :** PR polymerases are characterized by their exceptional thermostability, retaining over 90% of their activity even after prolonged incubation, such as for one hour, at a high temperature of 94°C.

**Assay Buffer (1X):** Tris-HCl (pH 8.8), (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>, KCl, 2mM MgSO<sub>4</sub>, Triton X 100 and Nuclease free BSA.



### Quality control assays:

- Absence of Nuclease Activity: 1 g of DNA digest was incubated with PR Polymerase with 1X assay buffer at 37°C and 72°C for 16 hours. The DNA visualized on 1% agarose gel showed sharp unaltered pattern.
- Absence of Nickase Activity: 1 g of super coiled DNA was incubated with PR Polymerase with 1X assay buffer at 37°C and 72°C for 16 hours. The DNA visualized on a 1% agarose gel showed unaltered pattern without nicking.
- Thermo Stability: PR Polymerase is a highly thermostable enzyme. The enzyme was incubated at 94°C for 1 hour and subsequently checked for activity. >90% activity was retained as determined by primer extension assay. Performance Test: PR Polymerase was extensively tested for PCR amplification of specific DNA fragments using both lambda DNA and human genomic DNA as templates. It was observed that for products 2Kb, further optimization is required. The conditions need to be optimized for each individual system. Increasing the enzyme concentration may increase the chance of primer degradation due to inherent 3' 5' exonuclease (proof reading) activity of the enzyme.

#### Storage: -20°C

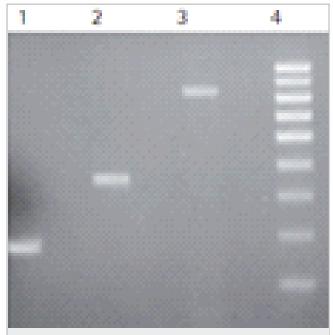


Fig 1: Amplification of human genomic DNA with ARMS primers (For ß-Thalassemia) using 1 unit of PR Polymerase, analysed on 2% agarose gel.

### Applications: >

 DNA Amplification: PR polymerase primarily used for DNA amplification, enabling the selective and exponential replication of specific DNA sequences. This application is fundamental to a wide range of downstream experiments.of Effi-

### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
0601700031730	MME17N	1 PPR Polymerase, 250 U (Enzyme 3 U / μl): 1 Vial:10XPR Polymease Assay Buffer: 1 vial

### XT-5 PCR system

XT-5 PCR system is a unique mixture of thermostable enzymes – Taq DNA Polymerase and PR (Proof Reading) Polymerase. This enzyme blend utilizes the powerful 5'-3' polymerase activity of Taq DNA Polymerase and the 3'-5' exonuclease mediated proof-reading activity of PR polymerase, resulting in high fidelity PCR products. This is particularly useful to amplify up to 5 kb target DNA from genomic DNA templates. A well-defined ratio of enzymes along with a highly optimized buffer system (XT-Polymerase buffers 5B) ensures specific PCR products. Assay buffer system 5B is best utilized for higher yields

**Reagents Supplied with Enzyme:** 1 vial of assay buffer 5B (Supplied as 10X

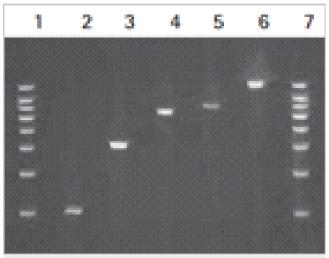
• XT-Polymerase Buffer 5B: (For higher yield) 1X Buffer composition: Tris-HCl (pH 9.0 at 25°C), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 1.5mM MgCl<sub>2</sub>.

Storage: Store enzyme and assay buffers at -20°C.

# **GeNei** TM

### Quality control assays: >

- Absence of Nuclease Activity: 1 g of DNA digest was incubated with XT-5 PCR System with 1X assay buffer at 37°C and 72°C for 16 hours. The DNA visualized on 1% agarose gel showed sharp unaltered pattern.
- ◆ Absence of Nickase Activity: 1 g of supercoiled DNA was incubated with XT-5 PCR System with 1X assay buffer at 37°C and 72°C for 16 hours. The DNA visualized on 1% agarsose gel showed unaltered pattern without nicking.
- ◆ Thermo Stability: XT-5 PCR system is most active at temperatures around 72°C and does not lose activity considerably even after prolonged incubation at high temperatures. Performance Test: XT-5 PCR system is tested for PCR amplification of fragment sizes 0.2 kb to 8 kb using both human genomic DNA and lambda DNA as templates. Sequence specific primers were used for amplifications.



**Fig 1:** Amplification of Lambda DNA using XT-5 PCR system. Target sequences of 2.0 to 10.0 kb were amplified using sequence specific primers and 1.5 units of XT-5 PCR system and analysed on 1% agarose gel

### Applications:

- Useful for high efficiency PCR amplifications. The products obtained can be used for gene cloning and other genetic manipulations.
- Ensures higher yields of the amplified product with improved fidelity
- Screening PCR: High-throughput screening of

- samples for the presence or absence of specific DNA sequences.
- Genotyping: Analysis of genetic variations, such as single nucleotide polymorphisms (SNPs) or insertions/deletions, in a population.
- RT-PCR (Reverse Transcription PCR): For the amplification of complementary DNA (cDNA) from RNA templates, commonly used in gene expression studies.

### References:

- Prasad R, Çağlayan M, Dai DP, Nadalutti CA, Zhao ML, Gassman NR, et al. (December 2017). "DNA polymerase β: A missing link of the base excision repair machinery in mammalian mitochondria
- Subba Rao K, Martin GM, Loeb LA (October 1985). "Fidelity of DNA polymerase-beta in neurons from young and very aged mice". Journal of Neurochemistry

### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
0601800031730	MME18L	XT-5 PCR system 250 U, (3 U/µl) (Enzyme: 1 Vial; XT-5 10X Assay Buffer 5B: 1 Vial)
0601800051730	MME18J	XT-5 PCR system 1000 U, (3 U/μl) (Enzyme: 1Vial; XT-5 10x Assay Buffer 5B:2 Vials)

### XT-20 PCR system

### Description: >

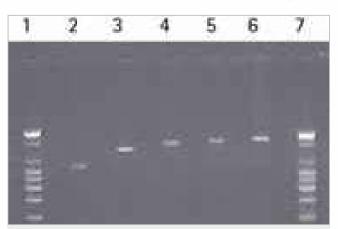
XT-20 PCR system is a unique mixture of thermostable enzymes - Taq DNA polymerase and PR (Proof Reading) polymerase. This enzyme blend utilizes the powerful 5'-3' polymerase activity of Taq DNA polymerase while the 3'-5' exonuclease mediated proof-reading activity of PR polymerase ensures high fidelity PCR products. This system is particularly useful to amplify long target DNA upto 20 kb from genomic DNA templates. A well-defined ratio of enzymes along with a set of highly optimized buffer systems (Assay buffers 20A and 20B) ensures specific PCR products. Assay buffer system 20A is

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optimized for amplifications of 8.0 kb to 12.0kb target sequences and assay buffer system 20B is optimized for 12.0kb to 20.0kb amplifications.

### Reagents Supplied with Enzyme: >

- 10X Assay Buffer XT-20A & 20B, 1 ml each with packsize of 100-250 U
- 10X Assay Buffer XT-20A & 20B, 2 ml each with pack size of 1000 U
- XT-Polymerase Buffer 20A: (For amplifications of 8.0 kb to 12.0 kb) 1X Buffer composition: Tris-HCl (pH 9.0 at 25°C), (NH4)2SO4 and 2 mM MgCl2.
- XT-Polymerase Buffer 20B: (For amplifications of 12.0kb to 20.0kb) 1X Buffer composition: Tris-HCl (pH 9.0 at 25°C), (NH4)2SO4 and 2.75 mM MgCl2.



**Fig 1**:Amplification of human genomic DNA using XT-20 PCR system. Specific target sequences of 8.0 to 22.0 kb were amplified using tPA gene sequence specific primers and 2.0 units of XT-20 PCR system and analysed on 0.6% agarose gel.

**Lane 1 & 7** - Super Mix DNA ladder

 Lane 2
 8055 bp

 Lane 3
 12026 bp

 Lane 4
 15036 bp

 Lane 5
 17703 bp

**Lane 6** - 22466 bp

### Quality control assays: >

- Absence of Nuclease Activity: 1 g of DNA digest was incubated with XT-20 PCR System with 1X assay buffer at 37°C and 72°C for 16 hours. The DNA visualized on 1% agarose gel showed sharp unaltered pattern.
- Absence of Nickase Activity: 1 g of supercoiled DNA was incubated with XT-20 PCR System with 1X assay buffer at 37°C and 72°C for 16 hours. The

DNA visualized on 1% agarose gel showed unaltered pattern without nicking.

• Thermo Stability: XT-20 PCR system is most active at temperatures around 72°C and does not lose activity considerably even after prolonged incubation at high temperatures.

### Performance Test:

 XT-20 PCR system is tested for PCR amplification of fragment sizes 8.0 kb to 25 kb using both human genomic DNA and lambda DNA as templates. Sequence specific primers were used for amplifications

### Related Product: KT76:

- Long PCR 20 kb Ampli kit is ideal for amplifications of longer target DNA fragments, upto 20kb, from genomic DNA templates using sequence specific primers. The kit provides all essential reagents to perform 50 individual reactions. Control DNA and sequence specific primers are included to perform 10 control reactions.
- XT-20 PCR system is a unique enzyme mix that combines the powerful 5'-3' polymerase activity of Taq DNA polymerase and the 3'-5' exonuclease mediated proofreading activity of PR polymerase, resulting in high fidelity PCR products.
- Highly optimised buffer systems (XT-Polymerase buffers 20A and 20B) guarantee better performance.
- Human genomic DNA with sequence specific primers to amplify 15.0 kb DNA fragment of tPA gene for 10 control amplification reactions are provided.

### Kit contents:

- XT-20 Polymerase
- XT-Polymerase buffers 20A and 20B.
- 30mM dNTP Mix
- Mineral Oil
- Control DNA with sequence specific primers to perform 10 control reactions.
- DNA Marker
- Instruction Manual

Storage: Store enzyme and assay buffers at -20°

# GeNei TM

### Applications:

- Useful for high efficiency PCR amplifications. The products obtained can be used for gene cloning and other genetic manipulations.
- Ensures higher yields of the amplified product with improved fidelity.
- Screening PCR: High-throughput screening of samples for the presence or absence of specific DNA sequences.
- Genotyping: Analysis of genetic variations, such as single nucleotide polymorphisms (SNPs) or insertions/deletions, in a population.
- RT-PCR (Reverse Transcription PCR): For the amplification of complementary DNA (cDNA) from RNA templates, commonly used in gene expression studies.

### References:

- Prasad R, Çağlayan M, Dai DP, Nadalutti CA, Zhao ML, Gassman NR, et al. (December 2017). "DNA polymerase ♦: A missing link of the base excision repair machinery in mammalian mitochondria
- Subba Rao K, Martin GM, Loeb LA (October 1985). "Fidelity of DNA polymerase-beta in neurons from young and very aged mice". Journal of Neurochemistry

### **Ordering Information:**

Cat. No	PI No.	Product Description
0601900031730	MME19L	XT-20 PCR system 250 U, (3 U/µl) (Enzyme: 1Vial; 10x Assay Buffer 20A: 1 Vial; 10X Assay Buffer 20B: 1 Vial)
0601900051730	MME19J	XT-20 PCR system 1000 U, (3 U/µl)(Enzyme: 1Vial; 10x Assay Buffer 20A: 2 Vials; 10X Assay Buffer 20B: 2 Vials)
0667600011730	KT76	GeNei™ Long PCR 20 kb Ampli Kit, 50 reactions

# MAGIC Amplification Solution (2.5X)

Amplification of templates with high GC content is difficult due to its robust secondary structure leading to inefficient template denaturation. MAGIC solution is a PCR enhancer that facilitates successful amplification of GC-rich sequences (up to 80%).

### Unique Features: >

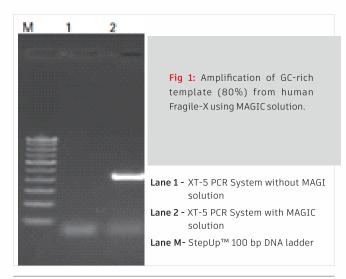
- A PCR enhancer facilitating amplification of GCrich templates (up to 80%)
- Does not adversely affect normal amplification reactions
- Does not alter fidelity of PCR enzymes
- Can be used with Taq DNA Polymerase as well as Long PCR Polymerase systems

### Related Product: KT92:

The kits include all the essential reagents required for standard amplifications along with a unique MAGIC solution. MAGIC solution is a unique blend that enhances PCR by eliminating secondary structure denaturation-related limitations caused by GC-rich template, and thereby ensuring specific amplification of such products. MAGIC solution can be used in combination with both Taq DNA Polymerase as well as XT-5 PCR System. Control DNA with primers that amplify a 600 bp fragment having 75 % GC-rich sequence, is supplied for 10 control reactions.

### Kit contents:

- Taq DNA polymerase and Assay Buffer
- 2.5X MAGIC Amplification Solution
- 10 mM dNTP Mix
- Control DNA and Primers for 10 reactions
- StepUp<sup>™</sup> 100 bp DNA Ladder (Ready to use)
- Instruction Manual



### Applications: >

- Amplification of GC-rich (up to 80%) templates
- Amplification of repeat sequences
- Multiplex PCR amplification from GC-rich templates

### References:

- Sarkar et al; Nucleic Acids Research, Vol. 18, No. 24; 1990. Formamide can dramatically improve the specificity of PCR
- Frey et al; Nature Protocols 3, 1312 1317 (2008). PCR-amplification of GC-rich regions: 'slowdown PCR'
- Saiki, R.K. et al. (1988) Science 239, 487-494.
- McConlogue, L. et al. (1988) Nucl. Acids Res. 16, 9869.
- Haqqi,T.M. et al. (1988) Nucl. Acids Res. 16, 11844. 6. Grandy,D.K. et al. (1989) Proc. Natl. Acad. Sci. USA 86, 9762-9766.
- Sommer,S.S. et al. (1990) In PCR Protocols: A Guide to Methods and Applications. Academic Press, pp. 197-205.
- Sarkar, G. and Sommer, S.S. (1989) Science 244, 331-334.
- Hung,T. et al. (1990) Nucl. Acids Res. 18, 4953.

### **Ordering Information:**

Cat. No	PI No.	Product Description
0653880011730	FC58L	MAGIC Amplification Solution (2.5X), (for GC-rich template), 1ml
0669200011730	KT92	GeNei™ MAGIC Amplification Kit 1 (for GC-rich template) Using Taq DNA Polymerase, 50 Reactions

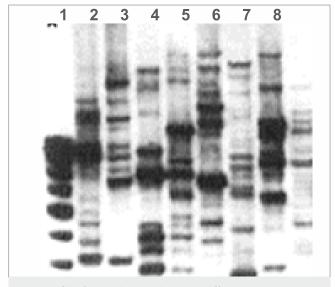
### **RAPD** primer sets

RAPD (Random Amplified Polymorphic DNA) is a polymorphism assay which is based on the amplification of random DNA segments using sets of primers of arbitrary nucleotide sequence. In theory, the primer anneals to many regions of the genome simultaneously. However, geometric amplification only occurs in those regions in which the 3' end of the annealed primers face one another on opposite strands and are no more than 3 Kb apart. Thus, the technique essentially scans a genome for these small inverted repeats and amplifies intervening DNA sequences of variable length. The RAPD Primer Set is designed for different organisms. There are five different primer sets available for bacteria, fungi, plants, animals and humans. It enables the user to choose the appropriate primer set for fingerprinting.

### 1. RBa-D

SI .No	Name of the primer	Accession Numbers
1	RBa 1	AM911690
2	RBa 2	AM773311
3	RBa 3	AM773772
4	RBa 4	AM911679
5	RBa 5	AM911680
6	RBa 6	AM773778
7	RBa 7	AM773318
8	RBa 8	AM911681
9	RBa 9	AM911682
10	RBa10	AM911683
11	RBa11	AM911684
12	RBa 12	AM911685
13	RBa 13	AM750047
14	RBa 14	AM911686
15	RBa 15	AM911687
16	RBa 16	AM765831
17	RBa 17	AM750058
18	RBa 18	AM773319
19	RBa 19	AM911688
20	RBa 20	AM911689
21	RBa 21	AM911691
22	RBa 22	AM911692
23	RBa 23	AM911693
24	RBa 24	AM911694
25	RBa 25	AM750048

# **GeNei** TM



RAPD profile of B.subtilis & E.coli using 4 different primers showing polymorphism

Lane 1: 100bp Ladder

Lane 2 & 3-B.subtilis & E.coli DNA with Primer RBa 1Lane 4 & 5-B.subtilis & E.coli DNA with Primer RBa 2Lane 6 & 7-B.subtilis & E.coli DNA with Primer RBa 3Lane 8 & 9-B.subtilis & E.coli DNA with Primer RBa 4

# 1 2 3 4 5 6 7 8

RAPD profile of S.cerevesiae & Fusarium sp., using 4 different primers showing polymorphism

Lane 1 & 2-S. cerevesiae & Fusarium sp., DNA with Primer Rfu 1Lane 3 & 4-S. cerevesiae & Fusarium sp., DNA with Primer Rfu 2Lane 5 & 6-S. cerevesiae & Fusarium sp., DNA with Primer Rfu 3Lane 7 & 8-S. cerevesiae & Fusarium sp., DNA with Primer Rfu 4

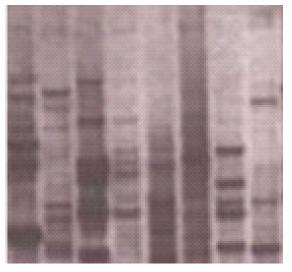
### 2. RFu-D

SI. No.	Name of Primer	Accession Numbers
1	RFu 1	AM911695
2	RFu 2	AM911696
3	RFu 3	AM911697
4	RFu 4	AM773320
5	RFu 5	AM911698
6	RFu 6	AM765822
7	RFu 7	AM911699
8	RFu 8	AM773321
9	RFu 9	AM773779
10	RFu10	AM765832
11	RFu11	AM911700
12	RFu 12	AM911701
13	RFu 13	AM911702
14	RFu 14	AM911703
15	RFu 15	AM765823
16	RFu 16	AM750053
17	RFu 17	AM911704
18	RFu 18	AM773322
19	RFu 19	AM773324
20	RFu 20	AM750056
21	RFu 21	AM911705
22	RFu 22	AM911706
23	RFu 23	AM750047
24	RFu 24	AM911707
25	RFu 25	AM911708

### 3. Rpl-D

SI. No.	Name of Primer	Accession Numbers
1	RPI 1	AM765819
2	RPI 2	AM750044
3	RPI3	AM773310
4	RPI4	AM773769
5	RPI 5	AM773770
6	RPI6	AM773771
7.8	RPI 7	AM773312
8	RPI 8	AM773773
9	RPI 9	AM773315
10:	RPI 10	AM750045
11	RPI 11	AM911709
12	RPI 12	AM773316
13	RPI 13	AM750046
14	RPI 14	AM773774
15	RPI 15	AM773775
16	RPI 16	AM773776
17	RPI 17	AM911710
18	RPI 18	AM765830
19	RPI 19	AM773777
20	RPI 20	AM773317
21	RPI 21	AM765820
22	RPI 22	AM911711
23	RPI 23	AM911712
24	RPI 24	AM765821
25	RPI 25	AM750054





RAPD profile of Horse gram and Cotton DNA using 4 different primers showing polymorphism

Lane 1 & 2 - Horse gram and Cotton DNA with Primer RPI1
Lane 3 & 4 - Horse gram and Cotton DNA with Primer RPI2
Lane 5 & 6 - Horse gram and Cotton DNA with Primer RPI3
Lane 7 & 8 - Horse gram and Cotton DNA with Primer RPI4

### Applications: >

- It plays a crucial role in gene mapping by identifying the position of linked genes in the chromosome which inherited together.
- It also detects any alteration in a sequence of DNA or any genetic oddity. It ascertains genes involved in genetic disorders.
- It is used to determine different characters in a DNA sequence which is used to distinguish between individuals, populations, or species.

### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
0692100251730	RBa-D	Bacterial Primer Set - Full, 25 Nos
0692300251730	RFu-D	Fungal Primer Set - Full, 25 Nos
0692800251730	Rpl-D	Plant Primer Set - Full, 25 Nos

### References: >

- Sambrook, J., Fritsch, E.F., and Maniatis, T., in Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY, Vol. 1, 2, 3 (1989).
- Austin, D.F. 1988. The taxonomy, evolution and genetic diversity of sweet potatoes and related wild species. In: P. Gregory (ed.).
   Exploration, maintenance, and utilization of sweet potato genetic resources. CIP, Lima, Peru. p. 27-60.

### Citations: >

- Multi-loci Molecular Characterisation of Endophytic Fungi Isolated from Five Medicinal Plants of Meghalaya, India Ranjan Kumar Bhagobaty#and S. R. Joshi\*
- Complication of Salmonella Bacteraemia in a Case of Treated Fungal Endophthalmitis J. Malathi,1 M. Sowmiya,1 Vikas Khetan,2 K. Lily Therese,1 and H. N. Madhavan1

# dNTPs mix, Set and Solution

Deoxynucleotide triphosphates (dNTPs) are essential components in the formation of DNA. These molecules consist of nucleoside triphosphates with deoxyribose as their sugar component. When they become part of the DNA structure during replication, they shed two of their phosphate groups. The family of dNTPs encompasses deoxyadenosine triphosphate (dATP), deoxythymidine triphosphate (dTTP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxyuridine triphosphate (dUTP). These dNTPs play a pivotal role as the fundamental building blocks in the intricate process of DNA synthesis.

### Quality control assays:

- Absence of nuclease activity: 1 μg of pUC 18 digest incubated with 10 mM EDTA at 37°C for 16 hrs showed sharp unaltered pattern on 1.2% agarose gel.
- Absence of nickase activity: 1 μg supercoiled plasmid DNA incubated with 10 mM of EDTA at 37°C for 16 hrs showed unaltered pattern without nicking on 1% agarose gel.
- Absence of RNase activity: 1 μg of total RNA incubated for 4 hours at 37°C with 10 mM EDTA in 20 μl reaction volume showed no degradation of RNA on 2% agarose gel.



### Related Products:

#### FC6Hl, FC7HL, FC8HL, FC8HL:

dNTP solutions are supplied in sterile double distilled water at 10 mM and 100 mM concentrations adjusted to neutral pH. These are supplied as individual products containing dATP, dCTP, dGTP and dTTP in separate tubes. dNTP solutions are used in applications that require highly pure dNTPs such as PCR, dideoxy sequencing, mutagenesis and cDNA synthesis

### Performance Test:

- dNTP solutions are tested for their performance in RAPD of rice DNA with 10 mer primer using Taq DNA Polymerase.
- Tested in long PCR amplifications of specific targets having sizes ranging from 2 to 20 kb from human genomic DNA.

All dNTP solutions supplied in 10 mM and 100 mM concentration.

Storage: -20°C

### Applications:

- DNA Replication: dNTPs are the substrates for DNA polymerases, the enzymes responsible for copying DNA during replication.
- PCR (Polymerase Chain Reaction): PCR is a widely used technique in molecular biology for amplifying specific DNA sequences. It relies on the use of dNTPs along with a DNA template, primers, and a DNA polymerase enzyme to generate multiple copies of a target DNA region.
- DNA Sequencing: dNTPs are used in various DNA sequencing methods, including Sanger sequencing and next-generation sequencing (NGS) technologies, to determine the sequence of nucleotides in a DNA molecule.
- DNA Labeling: dNTPs can be labelled with radioactive isotopes, fluorescent dyes, or other tags. Labelled dNTPs are used in techniques like DNA hybridization, Southern blotting, and fluorescence in situ hybridization (FISH) to detect and visualize specific DNA sequences.

### References:

- https://www.academia.edu/35906600/Second|\_Edition\_Methods\_in \_Molecular\_Biology\_1415
   Citations:
- Optimization of PCR reagents for amplification of microsatellites in oil palm M. Jayanthi\*, G. Sujatha and P.K. Mandal National Research Centre for Oil Palm Pedavegi, West Godavari District, Andhra Pradesh 534 450

### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>		
065230003173	0 FC23J	dNTP Mix, 10 mM (2.5 mM each), 1000 μl		
065240002173	0 FC24L	dNTP Mix, 10 mM (2.5 mM each), 4 x 200 μl		
065240004173	0 FC24J	dNTP Mix, 10 mM (2.5 mM each), 4 x 1000 μl		
0652200011730	D FC23HL	dNTP Mix, 100 mM (25 mM each), 100 μl		
065220002173	О FC23HJ	dNTP Mix, 100 mM ( 25 mM each), 1000 μl		
065230002173	0 FC23M	dNTP Mix, 40 mM (10 mM each), 1000 μl		
	dNTP	Set		
0651200011730	) Fc10	dNTP Set, 10 mM, 4 x 100 μl		
065120003173	O FC10J	dNTP Set, 10 mM, 4 x 500 μl		
065130002173	O Fc13L	dNTP Set, 10 mM, [5 x (4 x200 μl)] (20 vials)		
065100003173	O FC10HJ	dNTP Set, 100 mM, 4 x 250 μl		
	dNTP So	lution		
065060001173	0 FC6HL	dATP, 100 mM, 100 μl		
065070001173	O FC7HL	dGTP, 100 mM, 100 μl		
065080001173	0 FC8HL	dTTP, 100 mM, 100 μl		
065090001173	0 FC9HL	dCTP, 100 mM, 100 μl		

### Mineral Oil

### Description: >

Mineral oil is any of various colorless, odorless, light mixtures of higher alkanes from a mineral source, particularly a distillate of petroleum, as distinct from usually edible vegetable oils.

The name 'mineral oil' by itself is imprecise, having been used for many specific oils over the past few centuries. Other names, similarly imprecise, include 'white oil', 'paraffin oil', 'liquid paraffin' (a highly refined medical grade).

Most often, mineral oil is a liquid obtained from refining crude oil to make gasoline and other petroleum products. Mineral oils used for lubrication are known specifically as base oils. More generally, mineral oil is a transparent, colorless oil, composed mainly of alkanes and cycloalkanes, related to petroleum jelly. It has a density of around 0.8–0.87 g/cm3

Storage: Room temperature.

### Applications:

- Mineral oil is often used in PCR reactions to prevent the evaporation of reaction mixtures during thermal cycling.
- In reverse transcription reactions for cDNA synthesis, mineral oil can be used to create a barrier that helps maintain a stable reaction environment.
- Mineral oil is sometimes used in in situ hybridization reactions to prevent the drying of hybridization mixtures on tissue sections or cells.
- Mineral oil can be used in phenol-chloroform extractions during nucleic acid purification. The addition of mineral oil aids in the separation of phases and prevents the loss of aqueous samples during extraction steps

### References:

- "Mechanical properties of materials". Kaye and Laby Tables of Physical and Chemical Constants. National Physical Laboratory.
- Suwarno Darma, I.S.; Darma, I. S. (2008). "Dielectric properties of mixtures between mineral oil and natural ester.

### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
3601281001730	FC21L	Mineral Oil, 100ml

# DNA Diagnosis & Reagents

# WHITE SPOT SYNDROME VIRUS (WSSV)

### Description:

White spot syndrome virus (WSSV) infection has become the most dreaded disease problem affecting the farmed shrimp in India, causing mass mortalities and crop failures with huge economic losses for the farmers. White spot syndrome associated baculovirus is the causative agent of the disease. Affected shrimp develops white spots on the carapace and other parts of the body and mortality reaches upto 100% within 3 to 10 days after the first signs of the disease. Some do not succumb to the disease, but are carriers and are able to spread the pathogen. Therefore, it is very important to eliminate the carriers from the shrimp farming system. Till date, no treatment is known to control the disease. Hence, early diagnosis followed by suitable management practices is the only way in curtailing the disease. Conventional diagnosis fails to detect early stages of infection, and are also time consuming and less sensitive, whereas this kit is based on the principle of single-tube nested PCR (polymerase chain reaction), which is a powerful and sensitive diagnostic tool for identification of pathogens even at a very early stage (asymptomatic/carrier stage) of infection. This single-tube nested PCR involves an enzymatic hot start nested PCR in a single closed tube. In the

# GeNei TM

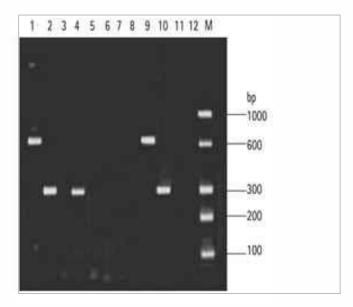
standard nested PCR, the second round PCR is setup using the first PCR product as template, which increases the chances of assay contamination by the product DNA. This is prevented by a single-tube nested PCR since all the amplification cycles take place in a single closed reaction tube not opened until the PCR is completed. In this assay, 3 sense and 1 antisense WSSV specific primers which produces either 3,2 or 1 PCR products (942bp, 525bp and 204bp) are used. Based on the severity of the infection, very high concentration of virus (105 viral particles) produces all the three fragments (942bp, 525bp and 204bp) whereas moderate to high concentration (103 viral particles) produces 2 fragments (525bp and 204bp) and low concentration of virus (10 to 200 viral particles) produces only the 204bp fragment. G

### Unique Features: >

- A single tube nested PCR reaction Less chance of contamination.
- Followed nested PCR principles enabling very high sensitivity of virus detection.
- Distinguishes the degree of severity of infection –
   Severe, Moderate and Low.
- Detects up to 10 virus particles in 3.5 to 4 hours
- · Fast and simple DNA extraction protocol
- Convenient aliquots of reagents Ease of work and reduced chances of contamination
- Sterile disposable grinders for easy sample processing

### Unique Features:

- DNA Extraction Buffer
- First PCR Pre-mix
- Nested PCR Pre-mix
- Taq DNA Polymerase
- Positive Control DNA
- Negative Control
- DNA Molecular Weight Marker
- Gel Loading Buffer
- Tissue Grinders, 1.5- & 0.5-ml vials



- Lane 1 Sample No.1 First PCR product Sample with high infection
- Lane 2 Sample No.1 Nested PCR product
- Lane 3 Sample No.2 First PCR product Sample with Low infection
- Lane 4 Sample No 2 Nested PCR product
- Lane 5 Sample No 3 First PCR product Sample with No infection
- Lane 6 Sample No. 3 Nested PCR product
- Lane 7 Negative control First PCR product
- Lane 8 Negative control Nested PCR product
- Lane 9 Positive control First PCR product
- Lane 10 Positive control Nested PCR product
- Lane 11 Reagent control First PCR product
- Lane 12 Reagent control Nested PCR product
- Lane M DNA Molecular Weight Marker

### References:

- Wang, HC; Hirono, I; Maningas, MBB; Somboonwiwat, K; Stentiford, G; ICTV Report, Consortium (July 2019). "ICTV Virus Taxonomy Profile: Nimaviridae". Th., m,e Journal of General Virology
- The white spot syndrome virus DNA genome sequence
- Van Hulten, M. C. W.; J. Witteveldt; S. Peters; N. Kloosterboer; R. Tarchini;
   F. Fiers; H. Sandbrink; R. K. Lankhorst & J. M. Vlak (2001)
- Chen, S. N. (1995). "Current status of shrimp aquaculture in Taiwan.". In C. L. Browdy; J. S. Hopkins (eds.)

### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
0680400011730	WSSV25	WSSV Detection Kit, (25 tests)
0680500051730	WSSV100A	WSSV Detection Kit, 100 tests with Gel electrophoresis consumables
Single Tube W	SSV Detection	on Kit
0680300011730	STWSSV50	Single Tube WSSV Detection Kit, 50 tests

### GeNei<sup>™</sup> Amplification Reagents Set for Malarial Parasites

### Description:

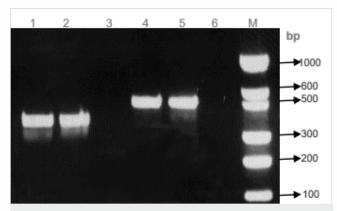
Malaria is an acute parasitic disease that kills an estimated 3 million people per year, mainly children, in developing countries. Global resistance to malaria is caused by insecticide resistance, spread of antimalarial drug resistance and increased movement of population. Early detection as well as species identification is a must for accurate treatment and follow-up of the disease. The classic diagnostic procedure remains as microscopic examination of Giemsa-stained blood smears that allows both the detection and identification of Plasmodium species. However, it is a technique that depends on experience and subjectivity of the observer. It is time-consuming, labor intensive and has limited sensitivity when parasitaemia is low. Serological tests are available, but they cannot always be used to distinguish between past and present infection because antimalarial antibodies may persist even after cure. Antigen detection methods are fast and simple to perform, but for patients with low levels of parasitaemia, the sensitivity decreases. The quantitative buffy coat (QBC) method is more sensitive, rapid and practical than microscopy, but is a relatively new technique and the limits of its sensitivity and specificity are still being explored. Reagents set is based on the principle of semi-nested multiplex PCR, a powerful and sensitive diagnostic tool and is used for the identification of Plasmodium falciparum and Plasmodium vivax species. It is a two-step sequential assay (nested PCR) and is based on the amplification of the sequences of the 18S small subunit ribosomal RNA (ssrRNA) gene. In first PCR reaction amplification observed at 750 bp using Plasmodium genus specific external primers. The second amplification is carried out with the same Plasmodium genus specific forward primer and two species-specific reverse primers for Plasmodium falciparum and Plasmodium vivax species, to amplify 395 bp and 499 bp amplification product respectively.

### Unique Features:

- Amplification set helps in the detection of Plasmodium falciparum and Plasmodium vivax by standard amplification methods.
- Reagents are packed in convenient aliquots to reduce the chances of contamination.
- Results can be obtained within 5-6 hours.

### Kit contents:

- I Amplification Pre-mix\*
- II Amplification Pre-mix\*\*
- Taq DNA Polymerase (3U/μl)
- Positive Control DNA 1
- Positive Control DNA 2
- 100bp DNA ladder (Ready to use)
- Proteinase K
- Gel Loading Buffer
- Lysis Buffer (For Blood)
- RT Wash Buffer I (concentrate)
- RT Wash Buffer II (concentrate)
- RT Elution Buffer
- Spin Columns
- Collection Tubes
- Dilution Buffer



Positive: Band at 395bp – P. falciparum Band at 499 bp - P. vivax Both 395 and 499 bp bands – mixed infection Negative: Absence of band

- **Lane 1** Sample1: Sample positive for P. falciparum
- $\textbf{Lane 2} \ \ \textbf{-} \ \mathsf{Sample 2: Sample positive for P. falciparum}$
- Lane 3 Sample 3: Negative sample
- Lane 4 Sample 4: Sample positive for P. vivax
- Lane 5 Sample 5: P. vivax positive control
- Lane 6 Reagent control
- Lane M Molecular weight marker



### References:

- Korenromp E, Williams B, de Vlas S, Gouws E, Gilks C, Ghys P, Nahlen B (2005). "Malaria attributable to the HIV-1 epidemic, sub-Saharan Africa". Emerging Infectious Diseases.
- Bartoloni A, Zammarchi L (2012). "Clinical aspects of uncomplicated and severe malaria". Mediterranean Journal of Hematology and Infectious Diseases.
- O'Brien C, Henrich PP, Passi N, Fidock DA (2011). "Recent clinical and molecular insights into emerging artemisinin resistance in Plasmodium falciparum"

### **Ordering Information:**

Cat. No	PI No.	Product Description
0670200011730	MAL25	GeNei™ Amplification Reagents Set for Malarial Parasites, 25 tests

# GeNei™ Amplification Reagents Set for Human Papilloma Virus

### Description:

Cancer of the uterine cervix is the most common malignant tumour in women world-wide and represents a major public health problem in southeast Asia. Human Papilloma virus (HPV) has emerged as a major pathogen associated with this disease. Human Papilloma Viruses are members of papova virus family and contain a double-stranded, circular DNA genome with a typical size of about 7900 bp. It has been shown from several studies that HPV infection is a good marker for women with cervical neoplasia and pre-cancerous lesion. Women persistently infected with certain "oncogenic" HPV types show a high rate of progression of dysplasia to invasive cancer of the cervix. Therefore, in best traditions of medical practice, the clinicians are obligated to seek out the presence of causal agents of the disease - the oncogenic types of HPV. Thus diagnosis of HPV infection may facilitate early identification of women at increased risk of developing cervical cancer. Pap screening program has been the mode of cervical screening for several decades. The biggest limitation of Pap test is the poor sensitivity (50-80%) and the need for frequent repetition. This kit

allows the detection of oncogenic HPV types. An amplification product of size varying between 230 – 270 bp is indicative of an infection with oncogenic HPV. The product size dependents on the type of HPV. This set detects oncogenic HPV types HPV 16, 18, 31, 33, 35, 45, 52b and 58. Due to its high specificity, the set allows quick screening in a variety of samples and an early detection of oncogenic genotypes.

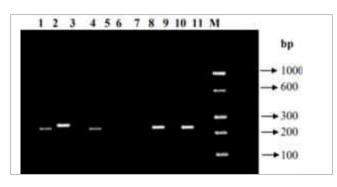
### Unique Features:

- This set greatly facilitates and hastens the detection of HPV by standard amplification methods.
- Fast and simple DNA extraction protocol.
- The reagents are packed in convenient aliquots to reduce the chances of contamination.
- The results can be obtained within 4-5 hours.
- Use of GeNei<sup>™</sup> HotStart Taq DNA Polymerse enhances the sensitivity and specificity.

### Kit contents:

- DNA Extraction Buffer
- Proteinase K
- HPV Amplification Mix
- ◆ GeNei™ HotStart Taq DNA Polymerase
- Positive Control DNA \*\*
- DNA Molecular Weight Marker (Ready To Use)
- Gel Loading Buffer
- Sample Collection Buffer
- Sample Wash Buffer
- ◆ Lysis Buffer II
- Wash Buffer I (Concentrate)
- RT Wash Buffer II (Concentrate)
- Elution Buffer
- Spin Columns
- Collection Tube

# **GeNei**



Lane 1 - Sample Positive for oncogenic HPV

Lane 2 - Sample Positive for oncogenic HPV\*

Lane 3 - Sample Negative for oncogenic HPV

Lane 4 - Sample Positive for oncogenic HPV

Lane 5 - Sample Negative for oncogenic HPV

Lane 6 - Sample Negative for oncogenic HPV

Lane 7 - Sample Negative for oncogenic HPV

Lane / - Sample Negative for oncogenic HPV

Lane 8 - Sample Positive for oncogenic HPV

Lane 9 - Sample Negative for oncogenic HPV

Lane 10 - Positive control

Lane 11 - Negative control

LaneM - DNA Molecular Weight Marker (Ready to use)

### References:

- Milner DA (2015). Diagnostic Pathology: Infectious Diseases. Elsevier Health Sciences.
- Meyers J, Ryndock E, Conway MJ, Meyers C, Robison R (June 2014).
   "Susceptibility of high-risk human papillomavirus type 16 to clinical disinfectants"
- Chen Z, Schiffman M, Herrero R, Desalle R, Anastos K, Segondy M, et al. (2011). "Evolution and taxonomic classification of human papillomavirus 16 (HPV16)-related variant genomes: HPV31, HPV33, HPV35, HPV52, HPV58 and HPV67"

### **Ordering Information:**

Cat. No	PI No.	Product Description
0670100011730	HPV25	GeNei™ Amplification
		Reagents Set for
		Human Papilloma
		Virus, 25 tests

# GeNei<sup>™</sup> Amplification Reagents Set for Mycobacterium Tuberculosis

Tuberculosis continues to be the leading cause of morbidity and mortality by an infectious disease worldwide. Effective treatment of TB requires the rapid detection of Mycobacterium tuberculosis. This set is based on the principle of single tube nested PCR targeting the IS6110 sequence that is present in multiple copies in the genome of the Mycobacterium tuberculosis complex. Studies have shown that highest sensitivity is obtained by using IS6110 nested PCR when compared to PCR targeting other regions. The diagnosis of TB is made by microscopy and culture. Direct Microscopy lacks sensitivity and specificity. Culture is more sensitive and is still the yardstick for diagnosis, but the time required and frequent negative results in paucibacillary specimens are the limitations. PCR is widely used for the detection of Mycobacterium tuberculosis in clinical specimens because the tests are rapid, sensitive, and specific

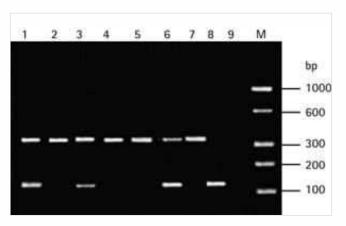
### Specifications:

- Detects specifically Mycobacterium tuberculosis complex present in samples.
- Based on the principles of nested PCR for increased specificity and sensitivity.
- Uses HotStart Taq DNA Polymerase for increased specificity.
- Carryover contamination prevention by dUTP-UDG system.
- Rapid Spin column-based DNA extraction for effective removal of PCR inhibitors from the sample.
- Results obtained in 4 hours.
- Convenient aliquots of reagents to reduce the chances of contamination.
- Internal control DNA provided for the identification of processed specimens containing substances that could inhibit PCR.
- Simulated positive controls included in the set.



### Kit contents:

- Tris Buffer (50X)
- Proteinase K
- Lysis Buffer I
- Lysis Buffer II
- Wash Buffer I (concentrate)
- Wash Buffer II (concentrate)
- Elution Buffer
- Spin Columns
- Collection Tubes
- I Amplification Premix
- II Amplification Premix
- HotStart Taq DNA Polymerase
- Uracil DNA Glycosylase (UDG)
- Positive Control DNA
- DNA Molecular Weight Marker
- Internal Control DNA Gel Loading Buffer



- **Lane 1 -** Sample 1 Sample Positive for Mtb
- Lane 2 Sample 2 Negative Sample
- Lane 3 Sample 3 Sample Positive for Mtb
- Lane 4 Sample 4 Negative Sample
- Lane 5 Sample 5 Negative Sample
- Lane 6 Sample 6 Sample Positive for Mtb
- **Lane 7 -** Sample 7 Negative Sample
- Lane 8 Sample 8 Positive Control
- Lane 9 Sample 9 Negative Control
- Lane M Molecular Weight Marker

### References:

• https://microbenotes.com/mycobacterium-tuberculosis

### **Ordering Information:**

Cat. No	PI No.	Product Description
0670300011730	MTB25	GeNei™ Amplification Reagents Set for Mycobacterium Tuberculosis , ( for 25 tests)
0670300021730	MTB50	GeNei™ Amplification Reagents Set for Mycobacterium Tuberculosis, (for 50 tests)

74

# GeNei

### **Products for RT-PCR**

# M-MuLV Reverse Transcriptase

### Description:

M-MuLV Reverse Transcriptase (M-MuLV RT) is a RNA dependent DNA polymerase that uses Single Stranded RNA or DNA as template. M-MuLV RT lacks endonuclease activity and has lower RNase H activity.

### Unit Definition:

One unit of M-MuLV Reverse Transcriptase is defined as amount of enzyme which incorporates 1 nanomole of [ 3H] dTMP into an acid insoluble product in 10 minutes at 37°C using poly (A) - oligo (dT) as template - primer.

### Storage Buffer:

20 mM Tris-HCl (pH 7,4), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% Igepal and 50% glycerol.

# Reagents Supplied with Enzyme:

5X Assay Buffer. 1X Assay Buffer: 50 mM Tris-HCl (pH 8.5 at 25°C), MgCl2 (optimised concetration), KCl (optimised concetration), and 10 mM DTT.

### Quality control assays: >

- Absence of Nuclease Activity: M-MuLV Reverse Transcriptase when incubated for 16 hours with 1 g of DNA digest at 37°C showed sharp unaltered pattern on 1% agarose gel.
- Absence of Nickase Activity: M-MuLV Reverse
   Transcriptase when incubated for 16 hours with 1
   g of supercoiled plasmid DNA at 37°C showed
   unaltered pattern without nicking on 1% agarose
   gel.

- ◆ Absence of Ribonuclease: M-MuLV Reverse
   Transcriptase when incubated for 4 hours with
   1 g E.coli RNA at 37°C showed no degradation of
   RNA as visualized on agarose gel.
- Performance Test: M-MuLV Reverse Transcriptase was tested in RT-PCR reaction of 500 bp fragment from GAPDH gene (cDNA) from total HeLa RNA and 2.1 kb fragment of polEgene from HeLa RNA.

Storage: Store enzyme and assay buffer at -20°C.



RT-PCR Amplified Products analysed. on 1% Agarose gel

### Applications:

- RT-PCR is used to prepare cDNA from eukaryotic mRNA, which lacks introns and can be inserted into prokaryotes. RT-PCR is used in monitoring the result of gene insertion and gene therapy. These procedures are supposed to show gene expression and code for a particular protein, hence translating specific types of mRNA sequence. This specific mRNA sequence can be analyzed using RT-PCR.
- Gene Expression: RT-PCR, we can amplify the minute mRNA sample and study the sequence of nucleotides, thus analyzing the gene expression. It is used in studying and identifying multidrugresistant genes and their expressions in pathogens.
- Species Identification: RT-PCR is used to identify viruses like HIV, SARS viruses, dengue viruses, HCV, etc. Besides, other microorganisms and even



higher organisms are identified by studying their rRNA and mRNA.

- Molecular Diagnostics: Diagnosis of different types of viral infection, bacterial infection, fungal and parasite infection, cancer cell, and genetic diseases are done using the RT-PCR technique in clinical laboratories.
- RT-PCR can detect and quantify tissue-specific mutant alleles. It can also detect any undesired changes in the mRNA sequence and unique mRNAs, which are produced only by the different types of cancer cells in our body.

### References:

- Ferris AL, Hizi A, Showalter SD, Pichuantes S, Babe L, Craik CS, Hughes SH (April 1990). "Immunologic and proteolytic analysis of HIV-1 reverse transcriptase structure
- Temin HM, Mizutani S (June 1970). "RNA-dependent DNA polymerase in virions of Rous sarcoma virus
- Hurwitz J, Leis JP (January 1972). "RNA-dependent DNA polymerase activity of RNA tumor viruses. I. Directing influence of DNA in the reaction". Journal of Virology.

### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>	
0601300051730s	MME13S	M-MuLV Reverse Transcriptase, 1000Units, 50 reaction, 20 U/ μl	
0601300061730	MME13L	M-MuLV Reverse Transcriptase, 5000Units, 250 reactions, 20 U/ μl	

# MMLV III Reverse Transcriptase

Reverse transcriptases (RTs) are enzymes using RNA as a template for DNA synthesis. Since the discovery in 1970, RTs have taken place in a common laboratory practice, which value hardly could be overestimated. RTs are applied to solve numerous tasks when RNA is a matter of concern, from pathogen detection or cloning to single-cell transcriptome analysis, allowing the possibility of working freely with fragile RNA molecules.

Among all known RTs, the reverse transcriptase from a Moloney Murine Leukemia Virus (M-MuLV RT) is one of the most scrupulously studied and well-known. M-MuLV RT was one of the first discovered RTs and served as a model for researching RTs properties and catalysis. Structure, catalysis, temperature optimum, thermostability, fidelity of DNA synthesis, processivity, optimal buffer composition, bypass of DNA damage, RNase H activity, strand transfer, and strand displacement activities – all biochemical properties of M-MuLV RT has been extensively investigated.

MMLV III Reverse Transcriptase (MMLV RTIII) is a ~ 70kDa RNA dependent DNA polymerase that uses Single Stranded RNA or DNA as template. MMuLV III RT lacks endonuclease activity and has lower RNase H activity.

Isolated from a recombinant source E.coli strain which harbours codon optimized gene from Moloney Murine Leukemia Virus

### Unit Definition:

One unit of MMLV III Reverse Transcriptase is defined as amount of enzyme which incorporates 1 nanomole of [3H] dTMP into an acid insoluble product in 10 minutes at 37°C using poly (A) - oligo (dT) as template - primer.

**Storage Buffer:** 20 mM Tris-HCl (pH 7,4), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% Igepal and 50% glycerol.

### Unique Features:

- ◆ RNase DNAse Free
- Lacks DNA endonuclease activity and has a lower RNase H activity.
- High purity/high quality control
- ◆ Thermostable reverse transcriptase active between42-50°C.

### Reagents Supplied with Enzyme:

5X Assay Buffer. 1X Assay Buffer:  $50 \, \text{mM}$  Tris-HCl (pH  $8.5 \, \text{at} \, 25^{\circ}$  C), MgCl2(optimised Conc.), KCl(optimised Conc.) and  $10 \, \text{mM}$  DTT.

Storage:-20°C

# **GeNei**

### Applications:

M-MLV Reverse Transcriptase has been used:

- ◆ To synthesize cDNA
- In quantitative Real Time-Polymerase chain reaction(RT-qPCR)
- In Reverse Transcription
- Preparation of cDNA libraries or for first strand cDNA synthesis
- For use in a 2-step RT-PCR assay
- For the synthesis of cDNA that is further used in cloning

### References:

#### M-MLV Reverse Transcriptase has been used:

- To synthesize cDNA
- In quantitative Real Time-Polymerase chain reaction (RT-qPCR)
- In Reverse Transcription
- Preparation of cDNA libraries or for first strand cDNA synthesis
- For use in a 2-step RT-PCR assay
- For the synthesis of cDNA that is further used in cloning
- Verma, I.M. (1977) Reverse Transcriptase in THE ENZYMES Vol.14A (P.D. Boyer, ed.) Pg.87-104 Academic Press, New York.
- Zagurski, R.J. Baumeister, K., Lomax, N., Berman, H.L. (1985) Rapid and easy sequencing of large double stranded DNA and supercoiled plasmid DNA. Gene Anal. Technologies 2:89-94.
- Roth,H. J. Tanese,N. and Goff, S.P. Purification and characterization of Murine retroviral reverse transcriptase Biochem. Biophysics. Acta 442:324-330
- George tzertzinis, Stanley Tabor, and Nicole M Nicols. Current protocols in Molecular Biology; Vol.3, Supplement 34, Unit 3.7.1-3.7.3.
- Sambrook, J. Fritsch, F.F., Maniatis, T. (1989) Reverse Transcriptase (RNA dependent DNA Polymerase), Molecular Cloning: A Laboratory Manual, Second Edition, 5.52-5.55.
- M-MuLV reverse transcriptase: Selected properties and improved mutants--Igor P. Oscorbin\* and Maxim L. Filipenko
- Yasukawa K., Mizuno M., Inouye K. Characterization of Moloney murine leukaemia virus/avian myeloblastosis virus chimeric reverse transcriptases.

### Ordering Information:

Cat. No	PI No.	<b>Product Description</b>
0604500011730	MME45S	MMLV III Reverse Transcriptase (100U/µl),1000 U
0604500021730	MME45L	MMLV III Reverse Transcriptase (100U/μl), 5000U
0604500031730	MME45B	MMLV III Reverse Transcriptase (100U/μl), 10000U

0604500041730 MME45HS MMLV III Reverse Transcriptase (200U/µl), 2000U

0604500051730 MME45HC MMLV III Reverse Transcriptase (200U/µl), 10000U

### M-MuLV RT-PCR Kit

### Description: >

The M-MuLV RT-PCR Kit provides a system for the detection, quantitation and analysis of mRNA. RNA is reverse transcribed to cDNA and amplified using the PCR process. The detection of the reverse transcription polymerase chain reaction (RT-PCR) product is done using gel electrophoresis. The kit includes all the necessary reagents for both cDNA synthesis and DNA amplification for 20 reactions. The kit uses M-MuLV (Moloney Murine Leukemia virus) Reverse Transcriptase which is a RNA dependent DNA polymerase. M-MuLV Reverse Transcriptase functions at 37°C and helps in removal of secondary structures in template RNA. The enzyme is not suitable for long cDNA synthesis (>5 kb). Total RNA and specific primers for GAPDH (glyceraldehyde 3 phosphate dehydrogenase) are supplied to carry out four control reactions. Template RNA concentration, primers, additives for high GC rich templates and cDNA to be used in the PCR reaction need to be optimized to get specific target amplification

### Unique Features:

- Two-step RT-PCR
- Can be used for prokaryotic and eukaryotic systems
- First-strand cDNA synthesis
- Amplification of full-length cDNA
- Kit includes all the necessary reagents for both cDNA synthesis and DNA amplification.

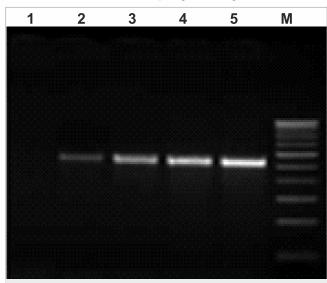
# GeNei TM

### Kit contents:

- Control Forward Primer
- Control Reverse Primer
- Oligo dT Primer
- Random Hexamer
- Total RNA
- M-MuLV Reverse Transcriptase
- 5X Assay Buffer M-MuLV
- Reverse Transcriptase Taq DNA Polymerase
- 10X Tag DNA Polymerase Assay Buffer
- RNasin
- 30mM dNTP Mix
- Nuclease Free Water
- DTT(100 mM)
- Dilution Buffer for Tag DNA Polymerase.

### Applications:

- Gene expression analysis
- Quantitative RT-PCR
- Cloning and gene expression studies.
- Detection of differential expression of gene
- Identification of multiple gene targets



Analysis of PCR Amplified 0.5 kb GAPDH gene using varying amounts of Total HeLa RNA, on agarose gel.

Lane 1 - 2 µg Total RNA (Negative Control)

Lane 2 - 100 ng Total RNA

Lane 3 - 250 ngTotal RNA

Lane 4 - 1 µg Total RNA

**Lane 5** - 2 μg Total RNA

Lane M - StepUp™ 100 bp DNA Ladder

### RTIII RT- PCR Kit

### Description:

The RTIII RT-PCR Kit provides a system for the detection, quantitation and analysis of mRNA. RNA is reverse transcribed to cDNA and amplified using the PCR process. The detection of the reverse transcription polymerase chain reaction (RT-PCR) product is done using gel electrophoresis. The kit includes all the necessary reagents for both cDNA

synthesis and DNA amplification for 20 reactions. The kit uses MMLV III Reverse Transcriptase which is a RNA dependent DNA polymerase.

MMLV III Reverse Transcriptase functions at 50°C and helps in removal of secondary structures in template RNA. The

enzyme is not suitable for long cDNA synthesis (>5 kb). Total RNA and specific primers for GAPDH (glyceraldehyde

3 phosphate dehydrogenase) are supplied to carry out 4 control reactions. Template RNA concentration, primers, additives for high GC rich templates and cDNA to be used in the PCR reaction need to be optimized to get specific target amplification.

### References:

- Verma, I.M. (1977) Reverse Transcriptase in THE ENZYMES Vol.14A (P.D. Boyer, ed.) Pg.87-104 Academic Press, New York.
- Zagurski, R.J. Baumeister, K., Lomax, N., Berman, H.L. (1985) Rapid and easy sequencing of large double stranded DNA and supercoiled plasmid DNA. Gene Anal. Technologies 2:89-94.
- Roth,H. J. Tanese,N. and Goff, S.P. Purification and characterization of Murine retroviral reverse transcriptase Biochem. Biophysics. Acta 442:324-330
- George tzertzinis, Stanley Tabor, and Nicole M Nicols. Current protocols in Molecular Biology; Vol.3, Supplement 34, Unit 3.7.1-3.7.3.
- Sambrook, J. Fritsch, F.F., Maniatis, T. (1989) Reverse Transcriptase (RNA dependent DNA Polymerase), Molecular Cloning: A Laboratory Manual, Second Edition, 5.52-5.55.

### **Ordering Information**

Cat. No	PI No.	Product Description
0667400011730	KT74	GeNei™ M-MuLV RT-PCR Kit, 20 reactions
2113080011730	KT308	RTIII RT-PCR Kit, 20 reactions

# One Step M-MuLV RT-PCR Kit

### Description: >

One Step Reverse Transcription PCR (RT-PCR) protocol is the method of choice when many RNA samples have to be analyzed simultaneously or if sample cross contamination is a certain risk. Both reverse transcription (RT) and PCR, excluding RNA template denaturation step, are set up and carried out in one reaction mix in a single tube. This is not only convenient, but also helps in eliminating sample to sample contamination. High sensitivity can be achieved using this protocol as all the synthesized cDNA is used as a template for the PCR step . The One Step M-MuLV RT-PCR kit provides a convenient format for highly efficient and specific RT-PCR using any RNA. The kit contains optimized components that allow both Reverse Transcription and PCR amplification to take place in what is commonly referred to as a "One Step Reaction".

The kit consists of two major components: RT-PCR Enzyme Mix and 2X RT-PCR Reaction Mix. The RT-PCR enzyme mix supplied with this kit is a mixture of Moloney Murine Leukemia virus (M-MuLV) Reverse Transcriptase, HotStart Taq DNA polymerase and a Fidelity Enzyme. The M-MuLV RT enzyme in the mixture can synthesize cDNA at a temperature range of 40-50°C, detect product upto 3 kb with varying amount of Total RNA (25 ng to 2 µg). The HotStart Tag DNA Polymerase included in the mix provides Hot Start PCR for highly specific amplification. During Reverse Transcription, HotStart Tag DNA Polymerase is completely inactive and does not interfere with the Reverse Transcription reaction. After Reverse Transcription by M-MuLV, reactions are heated to 95°C for five minutes to activate the HotStart Taq DNA Polymerase and to simultaneously inactivate the Reverse Transcriptase.

This procedure using HotStart Taq DNA polymerase eliminates extension from non specifically annealed primers and primer-dimers in the first RT cycle ensuring highly specific and reproducible PCR.

Although all the enzymes are present in the reaction mix, the use of HotStart Taq DNA Polymerase ensures the temporal separation of Reverse Transcription and PCR allowing both processes to be performed sequentially in a single tube. Only one reaction mix needs to be setup. No additional reagents are added after the reaction starts. 2X RT-PCR Reaction Mix, included in this kit, consists of an optimized single reaction buffer for Reverse Transcription and PCR , and includes MgSO4 to a final concentration of 2.5 mM which will produce satisfactory result in most cases, it also includes deoxyribonucleotide Triphosphates (dNTPs) and stabilizers. Sufficient reagents are provided for 25 or 100 amplification reactions of 50  $\mu$ l each.

### Unique Features: >

- Fast and convenient
- Avoids contamination
- Can be used for prokaryotic and eukaryotic system
- High sensitivity

### Kit contents:

- RT-PCR Enzyme Mix
- ◆ 2X RT-PCR Reaction Mix
- Water (DNase and RNase free)

RT-PCR amplified products of ß-actin gene from different no of HeLa and MCF-7 cells using One Step Reverse Transcription PCR (RT-PCR)kit analyzed on 1% agarose gel.

### Applications:

- Gene expression analysis
- Quantitative RT-PCR
- Cloning and gene expression studies.
- Detection of differential expression of gene
- Identification of multiple gene targets

### References:

 Verma, I.M. (1977) Reverse Transcriptase in THE ENZYMES Vol.14A (P.D. Boyer, ed.) Pg.87-104 Academic Press, New York.

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- Zagurski, R.J. Baumeister, K., Lomax, N., Berman, H.L. (1985) Rapid and easy sequencing of large double stranded DNA and supercoiled plasmid DNA. Gene Anal. Technologies 2:89-94.
- Roth,H. J. Tanese,N. and Goff, S.P. Purification and characterization of Murine retroviral reverse transcriptase Biochem. Biophysics. Acta 442:324-330
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- Sambrook, J. Fritsch, F.F., Maniatis, T. (1989) Reverse Transcriptase (RNA dependent DNA Polymerase), Molecular Cloning: A Laboratory Manual, Second Edition, 5.52-5.55.

### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
0661700021730	KT117M	GeNei™ One Step M-MuLV RT-PCR Kit, 50 reactions
0661700051730	KT117L	GeNei™ One Step M-MuLV RT-PCR Kit, 100 reactions

### cDNAdirect™ Kit

### Description:

cDNAdirect Kit is an optimized procedure to synthesize firststrand cDNA directly from cultured mammalian cells without isolating RNA. The synthesized first-strand cDNA is ready to be used in PCR, RT-PCR and other downstream applications such as quantifying mRNA level from a small number of cells. In cDNAdirect Kit, cultured cells are washed in PBS to remove cell culture medium and extracellular contaminants. Cells are then incubated with specially designed Lysis Buffer at 75°C to rupture the cells and inactivate the endogenous RNases. The crude cell lysate is treated with DNase I to degrade the genomic DNA and is ready to use for first strand cDNA synthesis and PCR using either a one-step or two-step Reverse Transcriptase-PCR.

### Unique Features:

- No RNA isolation or purification required.
- Simple protocol and generates high quality cDNA for use in a variety of downstream applications.
- Kit is optimized for synthesizing cDNA from small number of cells.
- Synthesized cDNA is free of genomic DNA contamination.

- Single step procedure to recover cDNA in 30 minutes.
- Allows detection of rare transcripts.
- Compatible with wide range of mammalian cell types.

### Kit contents:

- 10X PBS
- cDNAdirect Lysis Buffer
- DNase I (RNase free)
- cDNAdirect RT mix
- M-MuLV III Reverse Transcriptase
- RNase inhibitor
- Random Hexamer
- Positive RNA control
- Positive control sense primer
- Positive control antisense primer
- Nuclease Free Water

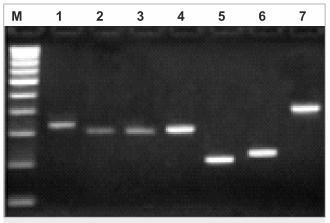


Fig 1: RT-PCR amplified products of different genes in NIH-3T3 cell line using cDNAdirect Kit.

Lane M - StepUpTM100 bp DNA ladder

Lane 1 - 326 bp fragment of RXRB gene

Lane 2 - 305 bp fragment of CASP9 gene

Lane 3 - 304 bp fragment of p53 gene

Lane 4 - 309 bp fragment of c-myc gene

**Lane 5:** - 208 bp fragment of PCNA gene

Lane 6 - 223 bp fragment of GAPDH gene

Lane 7 - 405 bp fragment of β-actin gene

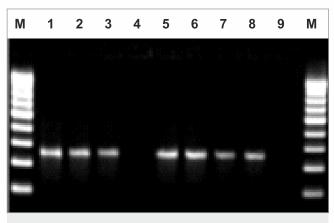
Lane 5: - 208 bp fragment of PCNA gene

Laile 5: - 206 up tragment of PCNA gene

**Lane 6** - 223 bp fragment of GAPDH gene

 $\textbf{Lane 7} \quad \textbf{-} \quad 405 \, \text{bp fragment of} \, \beta \text{--actin gene}$ 





**Fig 2:** RT-PCR amplification of -actin gene fragment (323 bp) from different number of HeLa & MCF-7 cells using cDNAdirect Kit.

### References:

- Hrdlickova, Radmila; Toloue, Masoud; Tian, Bin (January 2017). "RNA-Seq methods for transcriptome analysis.
- R, Kansal; K, Kuhar; I, Verma; Rn, Gupta; Vk, Gupta; Kr, Koundal (December 2008). "Improved and Convenient Method of RNA Isolation From Polyphenols and Polysaccharide Rich Plant Tissues.
- Temin H.M., Mizutani S. RNA-dependent DNA polymerase in virions of Rous sarcoma virus.

### **Ordering Information**

Cat. No	PI No.	Product Description
0667800021730	KT178S	cDNAdirect™ Kit, 20 preps

### **Random Hexamer**

### Description: >

Random hexamer is a mixture of random hexanucleotide primers that anneal to sequences throughout the target RNA, resulting in reverse transcription of both polyadenylated and non-polyadenylated RNAs.

The concentration of random hexamers in the reaction mixture is critical. As the ratio of hexamers to mRNA increases, the average length of the cDNA decreases. It is therefore essential to optimize the ratio of random primers and mRNA template in a series of pilot reactions. The average size of the first-strand cDNA generated in each of the pilot reactions should be measured by alkaline agarose gel electrophoresis and autoradiography using DNA markers of known size. When setting up a large-scale reaction to synthesize first-strand cDNA, use

the highest ratio of random primer to mRNA that generates cDNA of the maximum average length.

Random Hexamer Sequence: (CATG) 6

**Quantity Supplied:** A260 OD 7 g

Additional purification: PAGE Purified

Dissolving solvent: Sterile Distilled water

Storage: -20°C

### Applications: >

- DNA probe synthesis for use in Northern and Southern blots, and in situ hybridization applications.
- Partially degraded RNA samples.
- RNA without poly(A) tail such as ribosomal RNAs.
- RNA with strong secondary structure.

### References:

 Hansen, KD; Brenner, SE; Dudoit, S (2010). "Biases in Illumina transcriptome sequencing caused by random hexamer priming.

### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
0692470071730	Rh6	Random Hexamer (6 mer), 7µg

# Oligo dT primer pd (T)

### References:

The repeated sequences of deoxy-thymidine nucleotide sequence is used as oligos. It is usually used as a primer for cDNA synthesis (reverse transcription). It initiates the synthesis of the first strand by annealing to the 3' end of poly(A). Also, this primer is not suitable for RNA lacking poly-A tail or degraded RNA.In reverse transcription, an oligo(dT) primer is first annealed to the poly(A) sequences universally present at the 3' end of nearly every mRNA by T:A base-pairing. The reverse transcriptase then extends from the annealed oligo(dT) primer along the mRNA template, resulting in the copying of the mRNA sequence into the cDNA sequence In reverse transcription, an oligo(dT) primer is first annealed to the poly(A) sequences universally present at the 3' end of nearly every mRNA by T:A base-pairing. The reverse transcriptase then extends from the annealed oligo(dT) primer along the mRNA template, resulting in the copying of the mRNA sequence into the cDNA sequence.

Sequence: 5' - TTT TTT TTT TTT TTT-3'

Appearance: White Lyophilizate.

**Dissolving Solvent:** Sterile Distilled Water

Storage: -20°C



### Applications: >

- RT-PCR, PCR
- For the construction of cDNA libraries for gene identification.
- DNA probe synthesis for use in Northern and Southern blots, and in situ hybridization applications.

### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
0690970071730	DT 18	Oligo dT primer pd (T)18, 7µg

### **CLONING AND EXPRESSION**

### pUC18

### Description: >

pUC18 DNA is a commonly used E.coli cloning vector. The high copy number plasmid is a double stranded circular DNA, 2686 bp in length. There is a 54 bp multiple cloning site (MCS) polylinker region that has unique sites for 13 restriction endonucleases. The MCS is inframe with lacZgene, allowing insertion screening in colonies by complementation.

The DNA is isolated from E.coli strain by alkaline lysis method and further purified by density gradient. pUC18 DNA has ampicillin (Amp) resistance gene as selectable marker. The DNA is supplied in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. pUC18 DNA is identical to pUC19 DNA except that the MCS region is inverted (nt397-454).

Storage: -20°.

### **Quality Control Assay**

-	
Analysis	Specification
Electrophoresed 1 μg of DNA on a 1% agarose gel	More than 80% of observed DNA is in the supercoiled form
Absorption ratio A260:A280	1.5 to 2.0
Cleavage pattern verification Using Restriction enzymes(Eco RI, HaeIII ,PvuII & Xba I )enzymes followed by electrophoretic analysis on 1 – 1.5% agarose gel.	Typical cleavage pattern with clear sharp bands without Using Restriction enzymes(Eco RI, smear.
Self incubation with Genei assay buffers for 16 hours at 37°C to verify absence of detectable levels of nuclease activity.	Supercoiled form without nicks or linear band as visualized on 1% agarose gel.
MCS orientation in pUC18 DNA verified enzymes followed by electrophoretic analysis on 2% agarose gel.	3 bands of molecular sizes 2364, 176 and 146 bp.



### References:

- Yanisch-Perron, C.; Vieira, J.; Messing, J. (1985). "Improved M13 phage cloning vectors and host strains: Nucleotide sequences of the M13mp18 and pUC19 vectors.
- Wang, Nam Sun. "Summary of Sites on pUC19". Department of Chemical & Biomolecular Engineering University of Maryland.

### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
1600270101730	MBV1S	pUC18, 10µg
1600270501730	MBV1L	pUC18, 50µg
1600170501730	MBV1CL	pUC18 (Cesium Chloride Purified), 50µg

### pUC19

### Description:

pUC19 DNA is a commonly used E.coli cloning vector. The high copy number plasmid is a double stranded circular DNA, 2686 bp in length. There is a 54 bp multiple cloning site (MCS) polylinker region that has unique sites for 13 restriction endonucleases.

The MCS is in frame with lacZ gene, allowing insertion screening in colonies by complementation. The DNA is isolated from E.coli strain by alkaline lysis method and further purified by cesium chloride density gradient. pUC19 DNA has ampicillin (Amp) resistance gene as selectable marker. The DNA is supplied in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. pUC19 DNA is identical to pUC18 DNA except that the MCS region is inverted (nt397-454).

Storage: -20°C

### References:

- Yanisch-Perron, C.; Vieira, J.; Messing, J. (1985). "Improved M13 phage cloning vectors and host strains: Nucleotide sequences of the M13mp18 and pUC19 vectors.
- Wang, Nam Sun. "Summary of Sites on pUC19". Department of Chemical & Biomolecular Engineering University of Maryland.

### **Ordering Information:**

Cat. No	PI No.	<b>Product Description</b>
1600470501730	MBV2L	pUC19, 50µg
1600370501730	MBV2CL	pUC19 (Cesium Chloride Purified), 50µg

### **Quality Control Assay**

Analysis	Specification
Electrophoresed 1 µg of DNA on a 1% agarose gel.	More than 80% of observed DNA is in thesupercoiled form.
Absorption ratio A260:A280	1.5 to 2.0
Cleavage pattern verification Using Restriction Enzymes (EcoR I, Pvu II, Xba I & Sma I ) enzymes followed by electrophoretic analysis on 1 – 1.5% agarose gel	Typical cleavage pattern with clear sharp bands without smear.
Self incubation with Genei assay buffers for 16 hours at 37°C to verify absence of detectable levels of nuclease activity.	Supercoiled form without nicks or linear band as visualized on 1% agarose gel.
MCS orientation in pUC19 DNA verified by digestion with EcoR I and Pvu II enzymes followed by electrophoretic analysis on 2% agarose gel.	3 bands of molecular sizes 2364, 232 and 90 bp.



### **pBR322**

### Description:

pBR322 is a commonly used E.coli cloning vector. The DNA is isolated from E.coli strain by alkaline lysis method and further purified by cesium chloride density gradient. The plasmid is double stranded circular DNA, 4361 base pairs in length. pBR322 has ampicillin (Amp) and tetracycline (Tet) resistance genes as selectable markers. pBR322 can be amplified with chloramphenicol. The DNA is supplied in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

### Storage: -20°C

### **Ordering Information**

Cat. No	PI No.	Product Description
1600670101730	MBV3S	pBR322, 10µg
1600670501730	MBV3L	pBR322, 50µg
1600570501730	MBV3CL	pBR322 (Cesium Chloride Purified), 50µg

### Quality control assays

		•
	Analysis	Specification
	Electrophoresed 1 μg of DNA on a 1% agarose gel.	More than 80% of observed DNA is in the supercoiled form.
	Absorption ratio A260:A280	1.5 to 2.0
	Cleavage pattern verification using EcoRI, Hae III & Not I enzymes followed by electrophoretic analysis on 1 – 1.5% agarose gel.	Typical cleavage pattern with clear sharp bands without . smear
	Self incubation with Genei assay buffers for 16 hours at 37°C to verify absence of detectable levels of nuclease	Supercoiled form without nicks or linear band as visualized on 1% agarose gel.

### References:

- Watson, N. (1988). "A new revision of the sequence of plasmid pBR322".
   Gene
- Balbás P, Soberón X, Merino E, Zurita M, Lomeli H, Valle F, Flores N, Bolivar F (1986). "Plasmid vector pBR322 and its special-purpose derivatives--a review

# GeNei™ Instant Cloning Kit

TA Cloning is a simple and efficient method for cloning of PCR products. The procedure exploits the terminal transferase activity of certain thermophilic polymerases including Taq DNA Polymerase. Taq DNA polymerase preferentially adds a single adenosine residue to the 3' ends of the double stranded molecule and thus most of the molecules amplified by Taq DNA Polymerase possess single 3'-A overhang. The use of a linearised T-vector with 3'-T overhangs on both ends allows direct high efficiency cloning of PCR products carrying 3'-A overhang.

This kit includes 2X INSTANT ligation buffer and high activity INSTANT T4 DNA ligase to enhance single overhang ligation. The ligation reaction can be carried out for 1 hour at room temperature (25°C). Ligation can be continued at 4°C overnight to obtain maximum number of transformants. (or can be frozen at -20°C till use)

Genei T vector is a high copy number vector with pMB1 origin of replication. In addition, the vector also has f1 origin from filamentous phage f1 for the preparation of ss-DNA. The vector carries Ampicillin resistance marker. The Multiple Cloning Site (MCS) is within the lac Z  $\alpha$  fragment that allows for bluewhite screening. The insert can be released by double digestion (Ref Fig 1). The cloning region is flanked by SP6 and T7 RNA Polymerase promoter sequences.

activity.

### Unique Features:

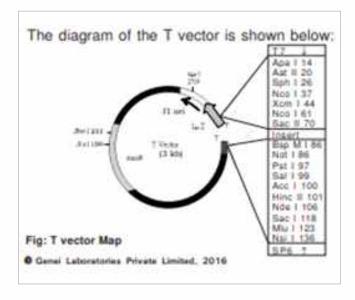
- 1 hour ligation at room temperature (25°C).
- No enzymatic manipulation of the product.
- Direct ligation of amplified product into T vector.
- Reduced background as self-ligation of the linearised Tyector cannot occur.
- Easy screening with blue white selection
- Insert can be released by double digestion with suitable enzymes.
- Sequencing of insert can be done using SP6 and T7 promoter primers.
- 30-60% clones expected depending on the insert.

### **Applications:**

 T-vector can be used to clone any double-stranded DNA fragment, including PCR products amplified by any DNA polymerase, as well as all blunt- and sticky-ended DNA species.

### References:

- Holton, T.A.; Graham, M.W (1991-03-11). "A simple and efficient method for direct cloning of PCR products using ddT-tailed vectors
- Clark, J. M. (1988). Novel non-templated nucleotide addition reactions catalyzed by procaryotic and eucaryotic DNA polymerases. Nucleic acids research, 16(20), 9677-9686.
- Hu, G. (1993). DNA Polymerase-catalyzed addition of nontemplated extra nucleotides to the 3' of a DNA fragment. DNA and cell biology, 12(8), 763-770
- Liu, Q., Dang, H. J., Wu, Y. H., Li, M., Chen, Y. H., Niu, X. L., ... & Luo, L. J. (2018). pXST, a novel vector for TA cloning and blunt-end cloning. BMC biotechnology, 18(1), 1-7.



### Kit Contents:

- Linearised T-vector
- ◆ Instant T4 DNA Ligase
- 2X Instant Ligation Buffer
- Nuclease Free Water
- Control DNA Insert
- Instruction Manual

### **Ordering Information**

Cat. No	PI No.	Product Description
1660630011730	KT63A	GeNei™ Instant Cloning Kit, 10 reactions

GeNei™ Competent Cells Preparation Kit (A), 50 reactions (Calcium Chloride method)

### Description:

Bacterial transformation is a process in which bacterial cells take up free/external DNA from the environment/surrounding medium. Cells which are capable of taking up DNA are said to be competent cells. The bacterial cells then acquire new traits because of the foreign DNA that is taken up by them. The ability to take up DNA efficiently by most bacteria is limited in nature. Natural competence is evident in B. subtilis, Streptococcus, Azotobacter, Haemophilus, Neisseria, and Thermus. Competence is a phenotype conferred by one or more proteins. Competence occurs late in the exponential phase of bacterial growth. The duration of competence varies from a few minutes in Streptococcus to hours in Bacillus. However bacterial cells can be artificially induced to take up DNA by treating them with calcium chloride. Culture of such cells that are capable of taking up DNA is said to be competent. The conditions required to produce competence vary from species to species. The efficiency of competent cells depends upon the strain, phase of growth arrested, competent cells preparation buffer etc. Transformation is a very basic technique that is

# **GeNei** TM

routinely used in a molecular biology lab. The purpose of this technique is to introduce a foreign plasmid DNA into bacteria and to use these bacteria to amplify the plasmid DNA

The kit makes use of a Calcium, Magnesium, Manganese chloride based buffer for the preparation of competent cells. The cations in the buffer neutralize the negative charges on the DNA backbone and cell membrane thereby allowing DNA to enter the cells. Competent cells of efficiency 107 per microgram of plasmid DNA can be obtained using this kit. The efficiency obtained varies with the strains and experimental conditions. Efficiency stated here is with respect to DH5 $\alpha$  strain transformed with pUC18 plasmid DNA.

### Highlights: >

- Competent cells of efficiency of 105 106 per microgram of plasmid DNA can be obtained using this kit
- Efficiency stated here is with respect to DH5α strain transformed with pUC18 plasmid DNA.
- The calcium chloride method, saves time and efforts

### Applications: >

- Competent cells have a wide variety of applications in Molecular Biology Research.
- Transformation experiments
- Preparation of cDNA or genomic libraries

### References:

- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed., 1.25-1.28. Cold Spring Harbor Laboratory Press, Cold Spring harbor, NY, USA.
- Dubnau, D., Provvedi, R. (2000). Internalizing DNA. Research in Microbiology, 151: 475-480
- Hanahan, D. Studies on Transformation of Eschrichia coli with Plasmids. J. Mol. Biol. 1983, 166, 557-580

### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
1660100011730	KT26	GeNei™ Competent Cells Preparation Kit (A), 50 reactions (Calcium Chloride method)

GeNei™ Competent Cells
Preparation Kit (B),
50 reactions (Modified
Calcium Chloride method)

### Description:

Bacterial transformation is a process in which bacterial cells take up free/external DNA from the environment/surrounding medium. Cells which are capable of taking up DNA are said to be competent cells. The bacterial cells then acquire new traits because of the foreign DNA that is taken up by them. The ability to take up DNA efficiently by most bacteria is limited in nature. Natural competence is evident in B. subtilis, Streptococcus, Azotobacter, Haemophilus, Neisseria, and Thermus. Competence is a phenotype conferred by one or more proteins. Competence occurs late in the exponential phase of bacterial growth. The duration of competence varies from a few minutes in Streptococcus to hours in Bacillus. However bacterial cells can be artificially induced to take up DNA by treating them with calcium chloride. Culture of such cells that are capable of taking up DNA is said to be competent. The conditions required to produce competence vary from species to species. The efficiency of competent cells depends upon the strain, phase of growth arrested, competent cells preparation buffer etc. Transformation is a very basic technique that is routinely used in a molecular biology lab. The purpose of this technique is to introduce a foreign plasmid DNA into bacteria and to use these bacteria to amplify the plasmid DNA

The kit makes use of a Calcium, Magnesium, Manganese chloride based buffer for the preparation of competent cells. The cations in the buffer neutralize the negative charges on the DNA backbone and cell membrane thereby allowing DNA to enter the cells. Competent cells of efficiency 107 per microgram of plasmid DNA can be obtained using this kit. The efficiency obtained varies with the strains and experimental conditions. Efficiency stated here is with respect to DH5 $\alpha$  strain transformed with pUC18 plasmid DNA.

### Unique Features:

- Easy methods, saves time and efforts Competent cells of efficiency 107 per μg of plasmid can be obtained using this kit.
- The efficiency stated here is with respect to DH5 α strain transformed per microgram of pUC 18 DNA.
- The efficiency varies with strain type and experimental conditions. High efficiency cells are particularly useful for difficult cloning.

### Applications: >

- Competent cells have a wide variety of applications in Molecular Biology Research.
- Transformation experiments
- Preparation of cDNA or genomic libraries
- High throughput cloning and protein expression.
- Sub cloning, TA cloning

### References:

- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed., 1.25-1.28. Cold Spring Harbor Laboratory Press, Cold Spring harbor, NY, USA.
- Dubnau, D., Provvedi, R. (2000). Internalizing DNA. Research in Microbiology, 151: 475-480
- Hanahan, D. Studies on Transformation of Eschrichia coli with Plasmids.
   J. Mol. Biol. 1983, 166, 557-580
- Holton, T.A.; Graham, M.W (1991-03-11). "A simple and efficient method for direct cloning of PCR products using ddT-tailed vectors
- Clark, J. M. (1988). Novel non-templated nucleotide addition reactions catalyzed by procaryotic and eucaryotic DNA polymerases. Nucleic acids research, 16(20), 9677-9686.
- Hu, G. (1993). DNA Polymerase-catalyzed addition of nontemplated extra nucleotides to the 3' of a DNA fragment. DNA and cell biology, 12(8),763-770.
- Liu, Q., Dang, H. J., Wu, Y. H., Li, M., Chen, Y. H., Niu, X. L., ... & Luo, L. J. (2018). pXST, a novel vector for TA cloning and blunt-end cloning. BMC biotechnology, 18(1), 1-7.

### **Ordering Information**

Cat. No	PI No.	Product Description
1660200011730	KT27	GeNei™ Competent Cells Preparation Kit (B), 50 reactions (Modified Calcium Chloride method)

### **GENOMIC DNA**

### **Calf Thymus DNA**

### Description:

pUC18 DNA is a commonly used E.coli cloning vector. The high copy number plasmid is a double stranded circular DNA, 2686 bp in length. There is a 54 bp multiple cloning site (MCS) polylinker region that has unique sites for 13 restriction endonucleases. The MCS is inframe with lacZgene, allowing insertion screening in colonies by -complementation.

The DNA is isolated from E.coli strain by alkaline lysis method and further purified by density gradient. pUC18 DNA has ampicillin (Amp) resistance gene as selectable marker. The DNA is supplied in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. pUC18 DNA is identical to pUC19 DNA except that the MCS region is inverted (nt397-454).

Storage: -20°.

### Quality control assays: >

**Nuclease Assay:** Absence of detectable levels of nuclease activity is verified by self incubation with Genei Buffers for 16 hours followed by gel electrophoresis.

**Storage:-** For continous use, keep the product refrigerated. For extended use, the product can be stored frozen in smaller aliquots

### Applications: >

- Used as a substrate for Deoxyribonucelase.
- Activated calf thymus DNA is a suitable substrate for unit estimation of DNA polymerases.
- Calf thymus DNA is used for studying the DNA binding agents, such as DNA binding anti-cancer agents.

### References: >

• Molecular complexes of calf thymus DNA with



various bioactive compounds: Formation and characterization-Chuye Ji <sup>1</sup>, Xin Yin <sup>1</sup>, Hongwei Duan<sup>2</sup>, Li Lianq <sup>3</sup>

### **Ordering Information**

Cat. No	PI No.	Product Description
5100680011730	CTS	Calf Thymus DNA, 1mg
5100780041730	CTL	Calf Thymus DNA, 4 x 1 mg

### Lambda DNA

### Description:

Lambda DNA is a double stranded linear molecule, 48502 base pairs in length, isolated from bacteriophage lambda by standard purification procedure. The phage is isolated from heat inducible lysogen, E.coli CI857 Sam7 and purified using cesium chloride block gradient. Lambda DNA is used as a substrate in restriction enzyme activity assays and in preparation of DNA molecular weight markers. The DNA is supplied in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

Storage: -20°C

### Quality control assays

Analysis	Specification
Cleavage pattern verification using Hind III, BamH I and EcoR I enzymes followed by electrophoretic analysis on 1 – 1.5% agarose gel	Typical cleavage pattern with clear sharp bands without additional bands and smear.
Absorption ratio A260 : A280	1.5 to 2.0
Self incubation with Genei assay buffers for 16 hours at 37°C to verify absence of detectable levels of nuclease activity.	Clear sharp band without additional bands and smear as visualized on 1% agarose gel.

### References:

- Casjens SR, Hendrix RW (May 2015). "Bacteriophage lambda: Early pioneer and still relevant
- Lederberg E (January 1950). "Lysogenicity in Escherichia coli strain K-12". Microbial Genetics Bulletin. 1: 5–8.; followed by Lederberg EM, Lederberg J (January 1953). "Genetic Studies of Lysogenicity in Escherichia Coli.

### **Ordering Information**

Cat. No	PI No.	Product Description
1600770601730	MBV6S	Lambda DNA, 60µg
1600773001730	MBV6L	Lambda DNA, 300µg

### **BACTERIAL STRAINS**

### E coli JM101

### Description:

JM101 is supplied in lyophilized form.

Genotype: supE, thi, (lac-proAB), [F', traD36, proAB, lac1qZM15]

### Features:

- JM101 strain carries the F' episome for pili allowing infection by filamentous phage such as M13 and by M13 helper phage for single stranded rescue from phagemid vectors.
- F' episome also has the lac Iq repressor gene producing 10-fold more lac repressor than is found in most strains, thereby ensuring more stringent repression of toxic proteins.
- F' episome has the lacIqm15 mutation which provides the complementation of the -galactosidase gene.
- This host allows blue / white colour selection assay for the plaques on plates supplemented with X-gal and IPTG.
- Used for methylation of plasmid DNA to allow efficient transfer to an hsdR+ E.coli host which is hsdM+

### **Growth Conditions:**

Temperature: 37° C Medium: Luria Bertini broth (LB) **Store: 4° C** 

# GeNei

### E coli JM109

### Description:

JM109 is supplied in lyophilized form.

Genotype: end A1, rec A1, gyr A96, thi, hsd R17 (rk - , mk +), rel A1, supE44, (lac - pro AB), [F', tra D36, proAB, lac Iq ZM15].

### Features:

- ◆ JM109 strain carries the F' episome for pili allowing infection by filamentous phage such as M13 and by M13 helper phage for single stranded rescue from phagemid vectors.
- F' episome also has the lac Iq repressor gene producing 10 fold more lac repressor than is found in most strains thereby ensuring more stringent repression of toxic proteins.
- F' episome has the IqZ M15 mutation which provides the complementation of the galactosidase gene allowing blue/white selection assay.
- Insert stability is ensured due to rec A- status. This strain also lacks the E.coli K restriction system thus preventing restriction of cloned DNA.
- end A1 mutation ensures quality and improved yield of plasmid DNA.
- Allows cloning with cleavage of transformed DNA by endogenous restriction endonucleases. DNA prepared from these strains can be used to transformrk + E.coli. strains.

### GrowthConditions: >

Temperature: 37° C Medium: Luria Bertini broth (LB) **Store: 4° C** 

### References: >

- Jeffrey H Miller A short Course in Bacterial Genetics CSHL Press 1992
- Yanisch-Perron, C.et al (1985), Gene, 33, 103.

### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
5101500011730	EC5	E.coli JM101, 1 vial
5101600011730	EC6	E.coli JM109, 1 vial

### **MUTAGENESIS**

# GeNei™ InSite PCR-Based Site Directed Mutagenesis Kit

### Description:

The Genei InSite PCR- Based Site Directed Mutagenesis Kit introduces point mutations and detects confirmed mutants with an unprecedented level of confidence of more than 95%. The DNA fragments to be mutated are cloned in a mutagenesis vector (pRVK2) and subjected to PCR. The protocol involves two steps of PCR reactions. In the first PCR, the mutation primer along with Primer I is used to introduce a unique restriction site Not I. The product of the first PCR is used as primer for second PCR for obtaining cloned vector with mutation. The second PCR products are transformed and colonies are screened by Not I digestion. Not I positive clones are mutants with more than 95% level of confidence.

### Unique Features:

- The mutants are predicted at more than 95% level of confidence.
- High fidelity XT-5 Polymerase ensures the absence of unwanted mutations, essential for protein expression studies.
- Easy detection of mutants by unique restriction enzyme digestion.
- Easy five step protocol.



### Kit Contents:

- Mutagenesis Vector
- Primer I
- XT-5 Polymerase
- 10 X Assay Buffer 5B
- dNTP mix.
- Not I Enzyme with 10 X Assay Buffer.( Not I)

### Applications:

- Generate point mutation, insertion, and deletion.
- Switch amino acids, delete or insert single or multiple amino acids.

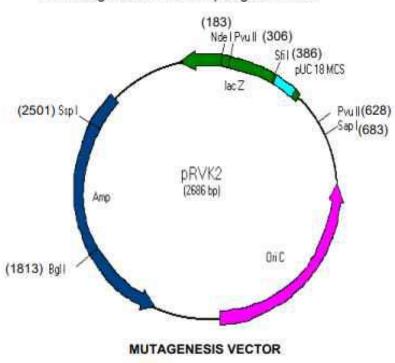
### References:

- Site-directed mutagenesis-Julia Bachman
- An efficient one-step site-directed and site-saturation mutagenesis protocol-Lei Zheng 1, Ulrich Baumann, Jean-Louis Reymond

### **Ordering Information**

Cat. No	PI No.	Product Description
0667100021730	KT71	GeNei™ InSite PCR- Based Site Directed Mutagenesis Kit, 10 reactions

### The mutagenesis vector map is given below:





#### DNA ISOLATION KITS SELECTION GUIDE

St. No.	Product Name	Starting Material	Yield	Time	Highlights
i.	PuresSol*** Plasmid Isolation Kit		High Copy: 10-15 µg (1.5 ml culture) Low Copy: 2-5 µg (3 ml culture)	45 min/24 preps	Fast and economical Suitable for large scale screening from E.coli Suitable for Restriction digestion, PCR, ligation etc.
2	GeneiPure™ Plasmid Purification Kit	Plasmid Culture	10-15 µg from 1.5-5 ml culture (High Copy) 10-15 µg from 10 ml culture (Low Copy)	50-60 min/ 12 preps	Purify 2-25kb plasmids Up to 80% recovery with >80% supercoiled form No genomic DNA or RNA Sequencing grade plasmid for downstream application like cloning, restriction digestion etc.
)	GeneiPure** Bacterial DNA Purification Kit	Bacterial Culture	10-15 µg from 0.5-1.0 ml (Gram -ve) 8-10 µg from 1-2 ml (Gram+ve)	4 hrs	Suitable for purifying up to S ml culture     High Yield     High quality DNA     suitable for restriction Digestion, PCR, RAPD etc.
4	UniFlex™ DNA Isolation kit.	Plant Tissue Bacterial Culture Yeast Culture Tissues Fecal matter	The yield varies according to the source: Blood: 8-10 µg Bacteria: 20-30 µg Feacal Matter: 5-6 µg Plant (leaves): 20-30 µg	90 mis	Fast and economical     Suitable for scale up     Suitable for restriction digestion     Purified DNA suitable for PCR, RAPD etc.
5	Gene-Pure** Yeast DNA Purification Kit	Yeast Culture	5-8 µg from 4-5 ml Culture	3 hrs	Unique Lysis Buffer and Lyticase provided for effective lysis
6//	GeneiPure <sup>***</sup> Plant Genomic DNA Purification Kit	Plant & Fungi (Different parts of plant & Seeds)	Rose leaves: 4-6 µg Mango Stem: 3-5 µg Algae: 1-5-2-5 µg Wheat leaves: 5-7 µg Cotton: 3-5 µg ample size: 100 mg	3 hrs.	Fast and Economical Two different Lysis buffer for processing different samples Spin filters included for clarification of Lysate Improved Lysis buffers for high yield and purity from variety of plant species and their parts
7	GeneiPure™ Mammalian Genomic DNA Purification Kit	Mammalian tissue ( fresh, frozen, burnt or decayed), Insects, Nematodes, Cell lines etc.	Liver: 30-50 µg Heart: 5-10 µg (Mouse) Mouse Tail: 5-7 µg Lung: 5-10 µg (Mouse) Sample size: 10-20 mg of tissue HeLa Cells (1-2x10* Cells): 8-9 µg Jurkat Cells (1-2x10* Cells):	2-4 hrs.	Fast, economical, suitable for wide variety for tissue samples. High quality DNA suitable for restriction digestion, PCR, RAPD etc.
8	GeneiPure™ Blood Genomic DNA Purification Kit	Blood, Serum, Buffy cost, Saliva, body fluids & Cell lines	Human Blood (0.2 ml): 4-6 µg	< 1hr or < 30 min	Fast, Economical and suitable for screening     experiments High yield from fresh or frozer blood Removes PCR inhibitors effectively Suitable for PCR, RAPO etc.
9	GeneiPure <sup>17</sup> Quick PCR DNA Purification Kit	PCR Product	Yield up to 80-90%	20min/6-12 prep	>95% removal of Primer DimerSuitable for sequencing, Cloning etc.
10	Gel Extraction Kit	Gel Extraction Kit	Yield up to 80-90%	20 min-30 min/6 preps	0.1-10 kb DNA can be purified DNA suitable for PCR, RAPD, restriction digestion etc.
11	GeneiPure™ Gel Extraction Kit		>70% Yield	20-30 min	Purified DNA suitable for PCR, RAPD, restriction digestion, cloning etc.



#### **SOLUTION BASED**

#### PureSol™ Plasmid Isolation Kit

#### Description:

PureSol™ Plasmid Isolation Kit contains ready to use reagents for high quality plasmid DNA isolation from overnight grown E.coli cultures. The method is very simple, rapid and cost effective, as it does not need any columns and can be carried out in a single tube per sample. PureSol™ Plasmid Isolation Kit is an efficient protocol for screening clones in a short time and obtaining sequencing grade DNA.

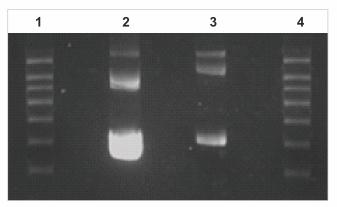
#### Unique Features:

- Minimum starting material required for isolation of pure and intact plasmid DNA.
- High recovery of DNA from both low copy number and high copy number plasmid.
- Rapid and simple isolation. No columns required.
- No/ Minimal genomic DNA contamination.
- · No RNA contamination.
- Good quality of DNA for subsequent use in molecular biology experiments like Restriction Enzyme Digestion, PCR, Ligation, Transformation and Sequencing.

#### Kit Contents:

- PureSol™ Solution A
- PureSol™ Solution B
- RNase A
- Lysozyme

Storage: RNase A and Lysozyme at -20°C. Buffers at room temperature.



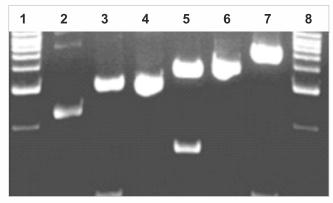
Purification of Plasmid DNA using PureSol™ Plasmid Isolation Kit.

Lane 1 - Supercoiled plasmid DNA Ladder

Lane 2 - High copy number plasmid

Lane 3 - Low copy number plasmid

Lane 4 - Supercoiled Plasmid DNA Ladder



**Lane1 -** StepUp™ 1 kb DNA Ladder

Lane 2 - Uncut plasmid (4 Kb)

Lane 3 - Plasmid digested with BamH I

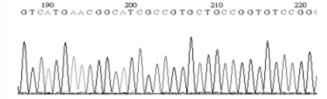
Lane 4 - Uncut plasmid (5 Kb)

Lane 5 - Plasmid digested with BamH I

**Lane 6** - Uncut plasmid (6Kb)

Lane 7 - Plasmid digested with Hind III

**Lane 8** - StepUp™ 1 Kb DNA Ladder



Electropherogram of the plasmid DNA purified using PureSol  $^{\text{TM}}$  Plasmid Isolation Kit.

#### References:

- Sambrook, J. et al., (1989) Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press.
- Ausubel, F.M. et al., eds. (1991) Current protocols in molecular biology, Wiley Interscience, New York

Cat. No	PI No.	Product Description
2116900021730	KT169	PureSol™ Plasmid Isolation Kit, 50 preps

#### UniFlex™ DNA Isolation Kit

#### Description:

Several applications of Molecular Biology require pure preparations of DNA isolated from various types of cells. Various techniques have been employed for nucleic acids isolation. The method used in the kit uses a phenol: chloroform extraction followed by ethanol precipitation of DNA. The method involves, firstly, removal of cellular proteins, in presence of salt, by a mixture of equal volumes of phenol: chloroform resulting in biphasic solution - an upper aqueous phase and a lower organic phase (mainly chloroform). Nucleic acid (RNA/DNA) partitions in the aqueous phase, while protein partitions in organic phase. In case of DNA extraction, RNase is added to remove RNA. In a last step, DNA is recovered from the aqueous phase by precipitation with 2-propanol or ethanol. Ethanol precipitation is a commonly used technique for concentrating and desalting nucleic acid (DNA or RNA) preparations in aqueous solution. The basic procedure is that salt and ethanol are added to the aqueous solution, which forces the nucleic acid to precipitate out of solution. The precipitated nucleic acid can then be separated from the rest of the solution by centrifugation. The pellet is washed in cold 70% ethanol then after a further centrifugation step the ethanol is removed, and the nucleic acid pellet is allowed to dry before being resuspended in clean aqueous buffer. The basic steps involved in the isolation of DNA with UniFlex DNA isolation Kit are: a) Sample collection and processing b) DNA isolation and c) DNA precipitation

The UniFlex DNA Isolation Kit provides a single solution for a simple, fast, reliable, reproducible, and convenient extraction of high amount of pure genomic DNA from various samples (bacterial, fungal, plant, and animal tissue). The kit uses specialized lysis solution and precipitation solution for purifying the DNA thus obtained.

#### Unique Features:

- Two step, effective and simple protocol for DNA isolation from wide variety of organisms and tissues.
- Solution based, giving high recovery. Does not involve any columns or beads.
- Can be easily scaled up to isolate DNA from larger amounts of tissues.
- Purified DNA is of high quality and ready for down processing like restriction analysis, PCR, Fingerprinting.

Sample	Starting Material (amount)	Yield (µg)
Plant leaves	100 mg	10-20
Green Gram seed	s 100 mg	5-10
Saliva	100 μl	2-3
Faecal pellet	100 mg	5-6
Urine	0.2 ml	~1
Mouse Tissue	100 mg	10-30
Semen	200 μl	5-7
Bacterial pellet	1ml	10-15
Fungal Pellet	100 mg	20-30
Blood	0.2ml	8-10

#### Kit Contents:

- ◆ RNAse<sup>™</sup>A
- UniFlex<sup>™</sup> Buffer I
- UniFlex<sup>™</sup> Buffer II
- UniFlex<sup>™</sup> Elution Buffer

#### Applications: >

- ➤ Using this kit, DNA can be isolated from the following:
- Bacterial cultures
- > Fungal and yeast cultures
- ➤ Animal tissues, human tissues, body fluids like semen, urine, saliva.
- ➤ Plant tissues: Isolated genomic DNA is ideal for use in common molecular biology procedures,



including PCR and qPCR, conventional restriction digestion, Southern blotting, Cloning, Finger printing analysis.

#### References:

- Chomczynski, P. & Sacchi, N. (1987). "Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction". Anal. Biochem. 162: 156–159. 2.
- Chomczynski, P. & Sacchi, N. (2006). "Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: Twentysomething years on". Nature Prot. 1 (2): 581–585.
- Molecular Cloning: A Laboratory Manual (Third Edition) by Joseph Sambrook, Peter MacCallum Cancer Institute, Melbourne, Australia; David Russell, University of Texas Southwestern Medical Center, Dallas
- Zeugin JA, Hartley JL (1985). "Ethanol Precipitation of DNA"Focus 7 (4):
   1–2. 5. Crouse J, Amorese D (1987). "Ethanol Precipitation: Ammonium Acetate as an Alternative to Sodium Acetate". Focus 9 (2): 3–5.

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2117000051730	KT170	UniFlex <sup>™</sup> DNA Isolation Kit, 100 preps

# GeNei™ Plant DNA Extraction Kit (for PCR amplification)

#### Description:

Plant DNA extraction kit is specifically designed to deal with carbohydrates, phenolics, and other compounds abundant in plant tissues. This kit yields high quality genomic DNA from tender leaf tissue at affordable cost. The DNA obtained can be directly used for PCR amplification and where lower quantity of DNA is required from several samples. The DNA is extracted from the leaf sample by dry ice/liquid nitrogen treatment followed by addition of Solution A (lysis buffer). Dry ice treatment makes the tissue vulnerable to lysis. Solution A lyses the plant cells by degrading cell walls and subsequent temperature treatment leads to a more complete lysis. DNA is concentrated in alcohol, dried, and rehydrated in Solution B. This kit yields enough DNA to serve as template for 7-15 PCR reactions depending upon the plant source and the resulting amplification patterns are indistinguishable from those generated from rigorous large-scale DNA extractions.

#### Unique Features: >

- Quick, Single-tube method
- No tissue homogenization required.
- Does not involve phenol extractions for many samples like sugarcane, rice, tomato etc.
- The resulting amplification patterns are indistinguishable from those generated from rigorous large-scale DNA extractions.

Note: We have tested in our lab various dicot & monocot leaf samples and have found reproducible RAPD results. However, good results were obtained only with very tender tissue. DNA yields were lower with tissues after certain degree of ageing.

#### Kit Contents:

- Solution A
- Solution B
- 3M Sodium Acetate

#### References:

- Chomczynski, P. & Sacchi, N. (1987). "Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction". Anal. Biochem. 162:156–159. 2.
- Zeugin JA, Hartley JL (1985). "Ethanol Precipitation of DNA"Focus 7 (4):
   1–2. 5. Crouse J, Amorese D (1987). "Ethanol Precipitation: Ammonium Acetate as an Alternative to Sodium Acetate". Focus 9 (2): 3–5.

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2102200051730		GeNei™ Plant DNA Extraction Kit (for PCR amplification), 100 preps

#### GeNei™ CTAB Plant DNA Extraction Kit

#### Description:

was initially used in bacteria (Jones, 1963) & later modified to obtain DNA from plants (Murray & Thompson 1980). The major difficulty in obtaining pure DNA samples from plants is that there are high concentrations of polyphenols, polysaccharides, terpenols and other substances. The CTAB method is

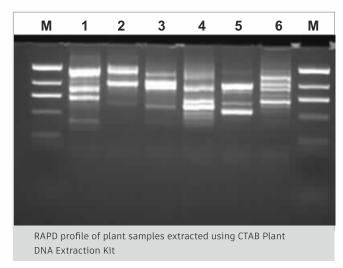


one of the most widely used methods of DNA extraction and ensures high yields of pure DNA since it complexes with nucleic acids.

This kit elaborates the protocol of isolating plant DNA using CTAB. When the initial NaCl concentration is lowered to ~ 0.5 M, CTAB forms an insoluble complex with nucleic acids. Polysaccharides, phenolic compounds & other enzyme inhibiting contaminants found in plant cells are efficiently removed in the supernatant as these do not precipitate under the conditions described. The nucleic acid – CTAB complex formed is soluble only in high salt TE solution. DNA is precipitated with 80% ethanol washes remove residual CTAB. Additionally beta mercapto ethanol is added because it is a strong reducing agent which can remove tannins and other polyphenols often present in the crude plant extract.

#### Kit contents:

- Solution A 40 ml RT
- Solution B 5 ml RT
- Solution C 50 ml RT
- Solution D 10 ml RT 1XTE



- $\textbf{Lane}\,\textbf{M}\quad \textbf{-}\; \text{QuantumTM}\;\; \text{PCR}\, \text{Marker}\, (\text{Low}\, \text{Range})$
- Lane 1-3 RAPD profile of Papaya leaf DNA with 3 different Plant RAPD primers
- Lane 4-6 RAPD profile of Tomato leaf DNA with 3 different Plant RAPD primers

#### Applications:

- RAPD analysis from plants.
- Zygosity and seed testing

#### References:

- C. Neal Stewart, Jr., and Laura E. Via. A Rapid CTAB DNA Isolation technique Useful for RAPD Fingerprinting and Other PCR Applications. BioTechniques 1993 article Vol. 14(5):748-749
- Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11-15.
- Doyle, J.J. and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue.

  Focus 12:13-15
- Jones A.S. and R.T. Walker, 1963, Isolation and analysis of the deoxyribonucleic acid of Mycoplasmamycoides var. Capri. Nature, 180, pp. 588-589.
- Mace ES, Buhariwalla HK, and Crouch JH (2003) A high-throughput DNA extraction protocol for tropical molecular breeding programs. Plant Mol Biol Rep 21: 459a-459hMurray M.G. and W.F. Thompson, 1980, Rapid isolation of high molecular weight plant DNA. Nucleic Acid Research, 8 (19), pp. 4321-4325

#### **Ordering Information**

Cat. No	PI No.	Product Description
2105500011730	Kt55	GeNei™ CTAB Plant DNA Extraction Kit (from leaves), 10 preps

#### GeNei™ Whole Blood DNA Extraction Kit (from fresh / frozen blood)

#### Description:

DNA extraction kit from whole blood is designed to provide quick, reliable, and reproducibly higher yields of genomic DNA from fresh blood as well as frozen blood. To avoid clotting, blood should be collected in EDTA coated collection vials/ tubes. The first step in the extraction procedure is the lysis of the red blood cells using Solution A, followed by lysis of the white blood cells and their nuclei using chaotropic Solution B. Finally, genomic DNA is concentrated and desalted by alcohol precipitation. The kit ensures extraction of highly pure DNA that can be used directly for PCR amplification and restriction digestion.

#### Unique Features: >

- No binding resin avoids shearing.
- No RNase treatment I Does not involve phenol extractions.
- Quick protocol

#### GeNei TM

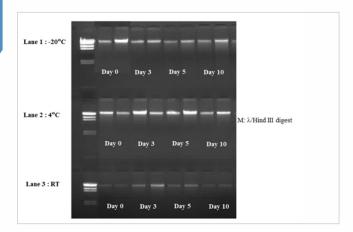
- Recovery from 300 µl of fresh Human Blood (8-10 µg)
- Recovery from 300 μl Frozen Human Blood (3 5 μg)

#### Kit contents:

- Solution A
- Solution
- Solution C

#### Application:

- Purified DNA can be used for downstream applications like PCR, Restriction Digestion.
- Diagnostics



Analysis of Human genomic DNA purified using Whole Blood Genomic DNA Extraction kit from blood samples (collected using EDTA as the anticoagulant), stored at 4C, -2OC and room temperature (RT) for 10 days, on 1% agarose gel

#### References:

- Albarino CG. V. Romanowski. Phenol extraction revisited: a rapid method for the isolation and preservation of human genomic DNA from whole blood. Mol Cell Probes 1994;8:423-7.2.
- Parzer S, Mannhalter C. A rapid method for the isolation of genomic DNA from citrated whole blood. The Biochemical journal 1991;273(Pt 1):229-31 from citrated whole blood. Biochem J 273Pt 11991:229-314.
- Pochi R, Genomic DNA. Subbarayan, Malancha( et al) Isolation of from Human Whole Blood. BioTechniques December 2002;33 SRC -GoogleScholar:1231-4.

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2102300011730		GeNei™ Whole Blood DNA Extraction Kit (from fresh / frozen blood), 50 prep <b>s</b>

#### GeNei™ Gel Extraction Kit, 100 preps

#### Description:

Gel Extraction Kit is designed to purify DNA fragments from standard or low melting agarose gels. The protocol is very rapid yielding ~70% DNA of high purity. The Gel Extraction Kit contains a specially formulated glass powder that binds single and double stranded DNA without binding DNA contaminants. The Kit contains enough reagents to perform 100 Preps.

#### Unique features: >

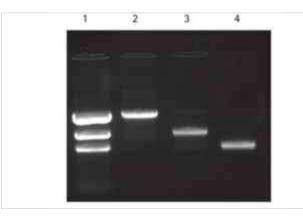
- Rapid 20 to 30 minutes.
- 70% of DNA is recovered.
- No alcohol precipitation.
- DNA obtained is of high purity.
- Can purify 0.5 kb 4 kb DNA fragment.

#### Kit contents:

- Silica
- Sodium Iodide
- Wash Buffer

#### Application: >

- Isolation of DNA fragment of interest from any grade of Agarose.
- · To remove impurities like RNAs and proteins.
- In rapid preparation of sequencing grade plasmid DNA and purification between enzymatic reactions, like restriction digests, dephosphorylation with CIP, ligations, or transformations.
- To remove residual phenol or chloroform.
- To remove unincorporated nucleotides from reaction mixtures.
- To remove primers from PCR products and excess linkers after ligations.
- · As an alternative to ethanol precipitation.



Purified pUC/Taq I fragments using KT02

Lane 1 - pUC18/Taq I Digest Lane 3 - 736 bp fragment
Lane 2 - 1444 bp fragment Lane 4 - 476 bp fragment

#### References:

- Molecular Biology Problem Solver: A Laboratory Guide. Edited by Alan S. Gerstein by Wiley-Liss, Inc. ISBNs: 0-471-37972-7 (Paper); 0-471-22390-5 chapter 7 page no 167-195.
- Current Protocols in Molecular Biology; Preparation and Analysis of DNA Vol1Supplement 58, Page 2.0.1-2.12.7.
- Molecular Biology Current Innovations and Future Trends- part I, Edited by Annette M.Griffin, Hugh G.Griffin, Page-39.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2100200051730	KT02	GeNei™ Gel Extraction Kit, 100 preps ( Silica - Solution Based)

#### **TriSol**

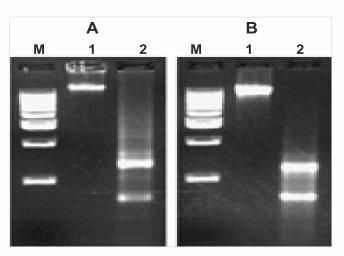
#### **Description:**

TRIsoln is ready to use single reagent containing guanidium thiocynate and phenol with propreitory components that helps in isolating total RNA. This reagent is based on the technology developed by Chomczynski and Sacchi. Tissue samples are lysed in TRIsoln, followed by addition of chloroform that separates RNA in the aqueous phase. TRIsoln comes with a dye which allows clear distinction of aqueous and organic phases. The aqueous phase contains RNA while the organic phase contains DNA and proteins that can be recovered by sequential precipitation. The isolated RNA/ DNA and proteins are suitable for all downstream applications.

Storage: 4°C

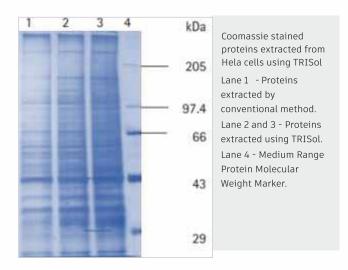
#### Applications: >

- ◆ Total RNA extracted can be used for downstream applications like RT-PCR. cDNA synthesis, Northern blotting, Invitro translation etc.
- DNA extracted can be used for restriction digestion, PCR amplification etc.
- Proteins obtained can be used for Western blotting, gene expression analysis etc.



Agarose gel showing simultaneous extraction of RNA from the same sample

 $\textbf{Isolation Kit.} (A) \, \text{Hela cells (B)} \, \text{Mouse Liver tissue}$ 



#### References: >

 Maniatis, T. Fritsch, E.F. & Sambrook, J. (1982), Molecular cloning: A laboratory manual p. 202, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Cat. No	PI No.	<b>Product Description</b>
2106481001730	FC64	GeNei™ TRIsoln, 100ml



#### **STET Lysis Solution**

#### Description:

STET Lysis Solution is a commonly used buffer for the lysis of bacterial cells such as E. coli for plasmid DNA isolation. Its provided in a ready-to-use solution format for rapid and efficient bacterial lysis. The buffer contains Sucrose, Triton X, EDTA and Tris which help in maintaining the osmotic pressure, cleaving the cell wall, chelating agent and buffering of the solution respectively in the plasmid isolation procedure.

#### Quality control assays:

Performance Test: Plasmid prepared from small culture volume (3-5 ml) using STET Lysis Solution. Quality of Plasmid obtained from this method is tested for Restriction Digestion by using ~ 1  $\mu$ g of plasmid DNA

#### References:

- Linke, Dirk (2009-01-01). "Chapter 34 Detergents: An Overview". In Richard R. Burgess; Murray P. Deutscher (eds.)
- Ji, Hong (2010-08-01). "Lysis of Cultured Cells for Immunoprecipitation". Cold Spring Harbor Protocols. 2010 (8): pdb. prot5466
- Brown, Robert B; Audet, Julie (2008-10-06). "Current techniques for single-cell lysis". Journal of the Royal Society Interface

#### Ordering Information

Cat. No	PI No.	<b>Product Description</b>
3100780501730	FC85M	STET Lysis solution, 50 ml

#### GeneiPure<sup>™</sup> DNA Purification Kits

#### GeneiPure™ Plasmid Purification Kit

#### Description:

A plasmid is a small, Circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA. A plasmid is an independent, self-replicating DNA molecule that carries only a few genes. Plasmids naturally exist in bacterial cells, and they also occur in some eukaryotes. Plasmids are easy to manipulate and isolate (Alkali lysis method or Boiling method). Scientists have taken advantage of plasmids to use them as tools to clone, transfer, and manipulate genes. Plasmids that are used experimentally for these purposes are called vectors. A foreign DNA fragment can be inserted into a plasmid vector, creating a recombinant plasmid. This plasmid can be introduced into a bacterium by the process of transformation. Since bacteria divide rapidly, they serve as factories to copy DNA fragments in large quantities. Vectors have selectable antibiotic marker, an origin of replication and a multiple cloning site (i.e site for various restriction endonucleases). Expression vectors contain promoter sequence that drives the expression of transgene. Besides this plamid vectors may contain genetic markers, epitope, protein purification tags and reporter genes. Thus, plasmids are important genetic engineering tools as they are used multiply, manipulate, study and express genes.

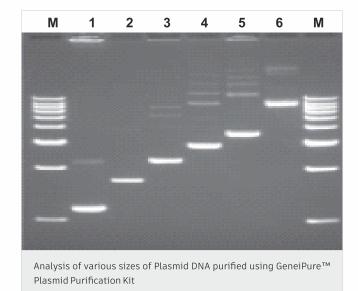
GeneiPureTM Plasmid Purification Kit is designed for rapid purification of high-quality plasmid DNA from bacterial cultures. Bacteria are lysed by the Lysis Buffer and the addition of high salt containing buffer facilitates neutralization and binding of DNA to the GeneiPureTM Column. Contaminants like metabolites, proteins, salts and other small molecules are removed by the two-step wash procedure with Wash Buffer I and Wash Buffer II. Pure Plasmid DNA is eluted using Elution Buffer underlowionic and slightly alkaline conditions.

#### Unique Features: >

- Purify high copy and low copy plasmids
- Plasmids upto 15kb can be isolated
- No detectable genomic DNA or RNA contamination
- Upto 80% recovery of DNA compared to conventional miniprep
- High recovery: High copy 10-15 μg of DNA from 1-5 ml bacterial culture . Low copy - 5-10 μg DNA from 5-10 ml bacterial culture.
- Fast and simple-12 preps/25 minutes.
- Culture volumes upto 10 ml can be processed owing to the high binding capacity of the new GeneiPure Column.

#### Kit Contents:

- RNase A (lyophilized)
- Solution G1
- Wash Buffer II (Concentrate)
- Elution Buffer
- Solution G2
- Solution G3
- Wash Buffer I
- GeneiPure™ DNA
- Spin Columns
- Collection Tubes



Marker (M)- StepUpTM 1kb DNA LadderLane 1- 2 Kb Plaskind DNA (Low Copy)Lane 2- 3 Kb Plaskind DNA (High Copy)Lane 3- 4 Kb Plaskind DNA (High Copy)Lane 4- 5 Kb Plaskind DNA (High Copy)Lane 5- 6 Kb Plaskind DNA (High Copy)

Lane 6 - 10 Kb Plaskind DNA (Low Copy)

#### Applications: >

 Purified plasmid prepared is suitable for all downstream applications like restriction digestion, transformation, PCR, sequencing etc.

#### References:

- Lezin G, Kosaka Y, Yost HJ, Kuehn MR, Brunelli L (2011). "A one-step miniprep for the isolation of plasmid DNA and lambda phage particles
- Serghini MA, Ritzenthaler C, Pinck L (May 1989). "A rapid and efficient 'miniprep' for isolation of plasmid DNA". Nucleic Acids Research
- Kovalenko SA, Tanaka M, Ozawa T (December 1994). "Simple methods for preparation of plasmid DNA yielding long and accurate sequence data". Nucleic Acids Research.

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2115200031730	KT152L	GeneiPure™ Plasmid Purification Kit, 50 preps

# GeneiPure™ Bacterial DNA Purification Kit

#### Description: >

GeneiPure™ Bacterial DNA Preparation Kit provides a rapid, simple method for the isolation of ready to use genomic DNA from gram positive & gramnegative bacteria. Bacterial samples are lysed using Proteinase K and Lysis Buffers and then loaded on to GeneiPureTM Column. DNA selectively binds to the membrane under high salt conditions while the contaminants pass through. Other impurities are completely removed by an effective wash step and pure nucleic acid is eluted in minimal volume of Elution Buffer. The purified genomic DNA is readyto-use in downstream applications like: PCR Cloning Restriction digestion.

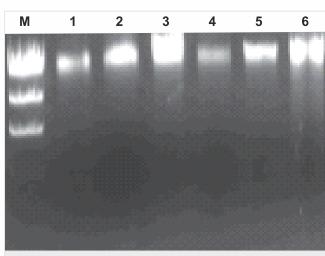
#### GeNei Th

#### Unique Features:

- Improved yield, upto 8-10 µg of DNA from 0.5-1 ml of E.coli culture and upto 5-10 µg of DNA from 1-1.5 ml Bacillus subtilis
- Unique lysis buffer optimized for Bacterial DNA Purification.

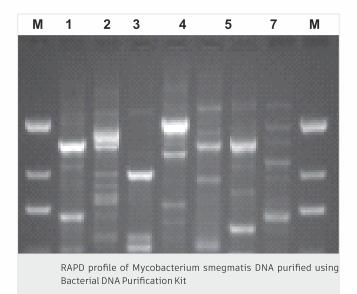
#### **Kit Contents:**

- Proteinase K1
- RNase A
- Lysozyme
- Bacterial Lysis Buffer
- Elution Buffer
- Lysis Buffer I
- ◆ Lysis Buffer II
- Wash Buffer I (Concentrate)
- Wash Buffer II (Concentrate)
- GeneiPureTM Columns
- Collection Tubes



DNA Purified from different Bacterial Strains using GeneiPure™ Bacterial DNA Purification Kit

Lane (M)-Lambda/Hind III MarkerLane 1-Staphylococcus Aureus 3ALane 2-Bacillus amyloliquefaciensLane 3-Bacillus globigiLane 4-Klebsiella pneumoniaLane 5-Neisseria siccaLane-Escherichia coli C



**Lane (M)** - Quantum<sup>™</sup> Low Range PCR Marker

Lane 1-7 - RAPD of DNA Purified from Mycobacterium Smegmatis using (different primers of bacterial primer set).

#### Applications:

- The purified genomic DNA is ready-to-use in downstream applications like: PCR Cloning Restriction digestion.
- Identification of new species of microorganisms.
- Whole genome sequencing.

#### References:

- A single protocol for extraction of gDNA from bacteria and yeast-Laurie Vingataramin and Eric H. Frost
- Heat Treatment of Bacteria: A Simple Method of DNA Extraction for Molecular Techniques Kuwait Medical Journal 2009; 41 (2): 117-122 Ali A Dashti1, Mehrez M Jadaon1, Abdulsamad M Abdulsamad2, Hussein M Dashti3 1 Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Health Science Center, Kuwait University, Kuwai.

Cat. No	PI No.	Product Description
2115900021730	KT159M	GeneiPure™ Bacterial DNA Purification Kit, 20 preps
2115900031730	KT159L	GeneiPure™ Bacterial DNA Purification Kit, 50 preps

#### GeneiPure™ Yeast DNA Preparation Kit

#### Description:

GeneiPure™ Yeast DNA Purification Kit provides a rapid, simple method for the isolation of ready to use genomic DNA from Yeast. Samples are lysed using Proteinase K and Lysis Buffers and then loaded onto silica membrane column. After a brief centrifugation, DNA is selectively bound to the membrane under high salt conditions while contaminants pass through. Other impurities are completely removed by effective wash steps and pure nucleic acid is eluted using the Elution Buffer.

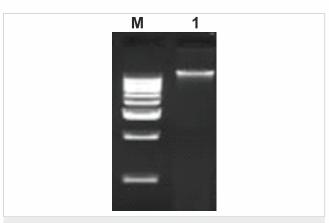
#### Unique Features: >

- Improved yield, upto 5-8 µg of DNA from 4-5 ml of Saccharomyces cerevisiae culture
- Unique Yeast Lysis Buffer with lyticase for effective and complete lysis.
- Purified genomic DNA is ready-to-use in downstream applications like: PCR, Cloning and Restriction digestion etc.

#### Kit Contents:

- Buffer
- Proteinase K
- Lyticase
- RNase A
- Lysis Buffer I
- ◆ Lysis Buffer II
- Wash Buffer I (Concentrate)
- Wash Buffer II (Concentrate)
- Elution Buffer
- GeneiPureTM Columns
- Collection Tubes

Sample	Amount	Yield (µg)
Saccharomyces	4-5mL culture	5-8
cerevisiae		



Agarose gel showing extraction of DNA from 1 ml of S.cerevisiae culture using yeast DNA preparation  $\,$  Kit

**Lane M -** StepUp $^{\text{TM}}$  1kb DNA ladder.

Lanes 1 - Yeast Genomic DNA

#### Applications:

- Host for Biopharmaceutical therapeutic proteins -Biopharmaceutical therapeutic proteins have been known since the 1980s and are produced by S. cerevisiae using modern biology techniques (e.g., genetic manipulation and monoclonal antibodies produced using hibridoma technology). Insulin and its analogues produced using S. cerevisiae are one example of a significant advance in the treatment of diabetes.
- ◆ Saccharomyces cerevisiae and S. pombe have been widely used in genetics and cell biology because they are simple eukaryotic cells that serve as models for all eukaryotes, including humans. Besides that, they allow researchers to investigate fundamental cellular processes such as the cell cycle, DNA replication, recombination, cell division, and metabolism.

#### References:

- Ling M, Merante F, Robinson BH. A rapid and reliable DNA preparation method for screening a large number of yeast clones by polymerase chain reaction. Nucleic acids research
- Akada R, Murakane T, Nishizawa Y. DNA extraction method for screening yeast clones by PCR. BioTechniques. 2000;28:668–670. 672, 674
- Amberg DC, Burke DJ, Strathern JN. Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual. 2005 Edition 2005.

Cat. No	PI No.	Product Description
2115800021730	KT158M	GeneiPure™ Yeast DNA Preparation Kit, 20 preps

#### GeNei TM

# Plant Genomic DNA Purification Kit

#### Description:

Several methods are available for extracting plant DNA. The protocol of isolating DNA using CTAB (Cetyltrimethyl ammonium bromide), a non ionic detergent, was initially used in bacteria (Jones, 1963) & later modified to obtain DNA from plants (Murray & Thompson 1980). Alternatively SDS (Sodium Dodecyl Sulphate) can be used to lyse of plant cells. The major difficulty in obtaining pure DNA samples from plants is the high concentration of polyphenols, polysaccharides, terpenols and other substances. The CTAB method is one of the most widely used methods of DNA extraction and ensures high yields of pure DNA since it complexes with nucleic acids. For Rapid and simple purification of plant DNA, spin column technology has been employed.

GeneiPure Plant Genomic DNA Purification Kit can be used for heterogeneous plant samples containing different metabolites like polyphenols, polysaccharides, or acidic components. Lysis Buffers have been optimized for processing some of the most common plant species. Optimized Wash Buffers completely remove proteins, RNA and other PCR inhibitors. Plant cells are homogenized and lysed with the Lysis Buffer containing chaotropic salts, detergents and denaturing agents. The lysate is cleared by centrifugation/ filtration this step removes the cell debris, polysaccharides and other contaminants. The supernatant is mixed with the Binding Buffer in order to facilitate optimum binding onto the GeneiPure TM Column. The contaminants are washed away using different buffers during the washing steps. DNA is eluted with a low salt Elution Buffer which is ready for further downstream applications.

#### Unique Features: >

- Silica membrane technology
- High yield from 5 μg 30 μg DNA
- DNA obtained can be directly used for cloning, PCR, restriction digestion etc.

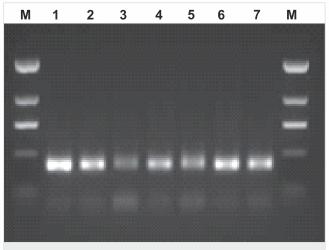
 Filter column supplied helps in clearing the lysate before loading onto the GeneiPure TM Column.

#### Kit Contents:

- RNase A
- Lysis Buffer
- Precipitation Buffer
- Binding Buffer (Conc)
- Wash Buffer I (Conc)
- Wash Buffer II (Conc)
- Elution Buffer
- GeneiPure™ Columns
- Filter column
- Collection Tubes

#### **Applications:**

- DNA Extraction from Plant tissues
- Purified DNA can be used in downstream applications like PCR, RAPD, Southern Blotting and other enzymatic reaction.



PCR of Plant DNA purified using GeneiPure  $^{\text{TM}}$  Plant Genomic DNA Kit from various plant sources with actin primers.

LlameeN5 - Lloi vaceRha heepef PCR Marker

Llame 16 - Rassentea feaf

Llamee27 - Rootste Rééalfs

Lane 3 - Mango stem

Lane 4 - Hibiscus flower

#### References:

- Plant genomic DNA isolation: An art or a science-Astha Varma, Harish Padh, Neeta Shrivastava.
- Extraction of DNA from plant tissues Scott O. Rogers & Arnold J. Bendich



#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>	
2115700021730	KT157M	GeneiPure™ Plant Genomic DNA Purification Kit, 20 preps	
2115700031730	KT157L	GeneiPure™ Plant Genomic DNA Purification Kit, 50 preps	

#### GeneiPure™ Genomic **DNA Purification** Kit- Mammalian tissues

#### Description:

GeneiPure<sup>™</sup> Genomic DNA purification kit -Mammalian tissues provides a simple and rapid method for purification of genomic DNA from mammalian tissues (liver, brain, heart etc). Lysis is achieved by incubation of the sample with Sodium Dodecyl Sulphate (SDS) and Proteinase K solution and incubating at 55C. Addition of chaotropic salt and ethanol to the lysate facilitates binding of the DNA to the membrane. Salts, metabolites and other macromolecules are removed by two subsequent washes with Wash Buffers. Pure genomic DNA is eluted using a slightly alkaline Elution Buffer.

#### Unique Features: >

- High recovery: upto 15-25 g of pure genomic DNA from 10 mg of tissue
- Sample size: 10-20 mg tissue
- No RNA contamination
- DNA purified can be used for many downstream applications like restriction analysis, PCR, sequencing, and other enzymatic reaction.

#### Kit Contents:

- Lysis Buffer I
- Lysis Buffer II
- Wash Buffer I (Concentrate)
- · Wash Buffer II (Concentrate)

- Elution Buffer
- Proteinase K
- RNase A
- GeneiPureTM Column
- Collection Tubes
- Instruction Manual



RAPD of DNA samples purified from decaying mouse tissue using GeneiPure™ Mammalian Genomic DNA Purification Kit

Yields of DNA obtained using GeneiPure Mammalian Genomic DNA

Material	Amount	Yield (ug)
LiverTissue (mouse)	15 mg	15-20
Spleen Tissue (mouse)	10 mg	15-20
Heart Tissue (mouse)	20 mg	5-10
Lung Tissue (mouse)	20 mg	5-10

#### References:

- Isolation of genomic DNA from mammalian cell-Cheryl M Koh.
- Isolation of High-Molecular-Weight DNA from Mammalian Tissues Using Proteinase K and Phenol.-Green MR, Sambrook J.

Cat. No	PI No.	Product Description
2115500031730	KT155L	GeneiPure™ Genomic DNA Purification Kit - Mammalian tissues, 50 preps

#### GeNei TM

#### GeneiPure™ Genomic DNA Purification Kit- Cells and Blood

#### Description: >

GeneiPure™ Genomic DNA Purification Kit - Cells & Blood has been designed to purify genomic DNA from fresh blood, serum, buffy coat, plasma or other body fluids. Blood treated with citrate, EDTA or heparin can be used. Cells are lysed in presence of chaotropic salt and Proteinase K. Binding to the GeneiPure Column is facilitated by addition of ethanol, this binding is reversible and is specific to nucleic acids. Contaminants like soluble macromolecules, salts and other metabolites are removed by wash buffers. Pure genomic DNA is eluted with a slightly alkaline elution buffer.

#### Unique Features:

- Silica membrane technology
- Sample size: Upto 200 µl of blood
- Recovery: 4-10 μg
- Ready-to-use DNA in 30 minutes
- Removal of PCR inhibitors

#### Applications:

Purified DNA can be used for downstream applications like PCR, Restriction Digestion, Cloning etc.

# M 1 2 3 4 5 6 7 8 M

Genomic DNA Purified from various Blood Samples & Cell Lines using GeneiPure™ Genomic DNA Purification Kit-Cells & Blood (1/50th of Eluate loaded)

Lane M - A / HindIII Digest

Lane 1 - Goat Blood (Heparin)

Lane 2 - Rabbit Blood (EDTA)

Lane 3 - Goat Frozen Blood (Heparin)

Lane 4 - Rabbit Frozen Blood (Heparin)

Lane 5 - HeLa Cells (Frozen)

Lane 6 - COS7 Cells (Frozen)

Lane 7 - MCF7 Cells (Frozen)

Lane 8 - NIH3T3 Cells (Frozen)



Amplification of DNA purified from various cell lines using GeneiPure™ Genomic DNA Purification Kit-Cells & Blood

**Lane M** - Quantum PCR marker (Low Range)

#### **Amplification using BRCA Primers**

Lane 2 - MCF (Mouse)
Lane 2 - COS7 (Monkey)
Lane 3 - HeLa (Human)
Lane 4 - NIH3T3 (Human)

#### **Amplification using RXRB Primers**

Lane 5 - MCF (Mouse)
Lane 6 - COS7 (Monkey)
Lane 7 - Hela (Human)
Lane 8 - NIH3T3 (Human)

- Molecular Biology Problem Solver: A Laboratory Guide. Edited by Alan S. Gerstein Copyright © 2001 by Wiley-Liss, Inc.ISBNs: 0-471-37972-7 (Paper); 0-471-22390-5 chapter 7 page no 167-195.
- Current protocols in molecular biology; preparation and analysis of DNA Vol 1 Supplement 58, Page 2.0.1-2.12.7.
- Molecular biology current innovations and future trendspart I, Edited by Annette M.Griffin, Hugh G.Griffin, Page 39.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2115600031730	KT156L	GeneiPure™ Genomic DNA Purification Kit - Cells and Blood, 50 preps

#### GeneiPure™ Quick PCR Purification Kit

#### Description:

The kit is designed for purification of PCR products by removal of primer, primer-dimers and low molecular weight DNA fragments generated by nonspecific amplification. DNA binds to silica membrane in presence of chaotropic salt present in the Binding Buffer. This mixture is applied onto silica membrane column. Contaminants like salts, soluble macromolecules, enzyme etc are removed by washing with an ethanolic Wash Buffer. Pure DNA is eluted using a slightly alkaline Elution Buffer or water.

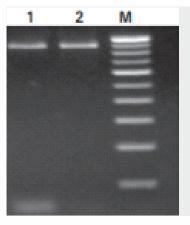
#### Unique Features:

- Complete removal of primers and primer-dimers
- Optimized Wash Buffers for greater purity of DNA
- ◆ Rapid DNA purification 6 Preparations/15 minutes

#### Kit Contents:

- Binding Buffer
- Wash Buffer I
- Wash Buffer II (Concentrate)
- Elution Buffer
- GeneiPure™ Columns
- 2 ml Collection Tubes
- Instruction Manual

#### Storage: Room temperature



Analysis of 0.9 kb PCR product purified using GeneiPure™ Quick PCR Purification Kit.

Lane 1 - Unpurified 0.9 kb PCR fragment

Lane 2 - Purified 0/9 kb PCR fragment

**Lane M** - StepUp<sup>™</sup>100 bp DNA Ladder

#### Applications: >

 Purified DNA can be used directly for downstream applications like sequencing, cloning, restriction digestion or any other enzymatic manipulation.

#### References:

- An efficient method for purification of PCR products for sequencing Hao Ma 1. Stephen Difazio
- Simple preparation method of PCR fragments for automated DNA sequencing.-Høgdall E, Boye K, Vuust J
- Rapid re-amplification of PCR products purified in low melting point agarose gels.-Zintz CB, Beebe DC

#### **Ordering Information**

Cat. No	PI No.	Product Description
2115300031730	KT153L	GeneiPure™ Quick PCR Purification Kit, 50 preps
2115300051730	KT153J	GeneiPure™ Quick PCR Purification Kit, 250 preps

#### Description: >

#### GeneiPure™ Gel Extraction Kit

Electrophoresis of DNA using polyacrylamide or agarose gels is one of the core techniques used in molecular biology. This method is used to separate, identify and purify DNA fragments. Agarose gels can be used to effectively separate fragments from 50 bp to several thousand bases in length by varying the porosity of the gel and application of current. Migration of DNA through the pores of gel depends upon the size and conformation of DNA. Electrophoresed DNA can be purified from gels by a number of methods such as: Electroelution, electrophoresis onto DEAE Cellulose/Nitrocellulose (NA45) paper, using  $\beta$ -Agarase (from Low Melting Agarose) or using glass beads/silica etc. GeneiPureTM Gel Extraction Kit provides a rapid method to extract and purify DNA fragments from standard or low melting agarose gels prepared in either Tris acetate (TAE) or Tris borate (TBE) buffer. DNA fragments ranging from 100bp to 10kb can be extracted and purified and DNA obtained can be directly used for downstream applications.

The kit utilizes a Gel Solubilization solution for melting the agarose gel containing DNA. Chaotropic salt present in it facilitates binding of DNA to the silica membrane. Contaminants like salts, and soluble macromolecules are removed by washing the column with Wash Buffer. Pure DNA is eluted using Elution Buffer

#### GeNei TM

#### Unique Features:

- No phenol-chloroform extraction or enzymatic digestion of agarose
- Rapid DNA purification 6 Preparations in 15 minutes
- High yield-upto 90% yield
- · Both TAE and TBE gels can be used
- Low Melting and Low EEO Agarose gels can be used
- Wide range of DNA fragments 100bp-10kb can be purified.

#### Applications: >

 DNA obtained can be used for various downstream applications like restriction digestion, PCR, sequencing.



**Fig 1:** Analysis of varying amounts of 8kb DNA fragment purified from agarose gel using GeneiPure™ Gel Extraction Kit

- Lane M StepUp™ 1 Kb DNA Ladder
- $\textbf{Lane 1} \quad \text{-} \quad 0.5\,\mu\text{g of 8kb fragment}$
- Lane 2 2.0 µg of 8kb fragment
- Lane 3 4.0 µg of 8kb fragment
- Lane 4 6.0 µg of 8kb fragment
- Lane 5 8.0 µg of 8kb fragment
- Lane 6 10 µg of 8kb fragment



Fig 2: Analysis of various size DNA fragments purified from agarose gelusing GeneiPure™ Gel Extraction Kit.

**Lane M** - StepUp<sup>™</sup>1Kb DNA Ladder

Lane1 - 1Kb fragment

Lane 2 - 3Kb fragment

Lane3 - 6Kb fragment

Lane 4 - 10Kb fragment

#### References:

- Recovery of DNA from Low-Melting-Temperature Agarose Gels: Organic Extraction.-Green MR, Sambrook J
- A rapid method for extracting DNA from agarose gels Author links open overlay panelMark Finkelstein 1, Robert H. Rownd 1

Cat. No	PI No.	<b>Product Description</b>
2115400021730	KT154M	GeneiPure™ Gel Extraction Kit, 20 preps
2115400031730	KT154L	GeneiPure™ Gel Extraction Kit, 50 preps

#### GeneiPure<sup>™</sup> Fungal Genomic DNA Purification Kit

#### Description:

GeneiPure<sup>™</sup> Fungal Genomic DNA Purification Kit can be used for heterogeneous Fungal samples containing different metabolites like polyphenols, polysaccharides or acidic components. Lysis Buffer has been optimized for processing some of the most common Fungal species. Optimized Wash Buffers completely remove proteins, RNA, & other PCR inhibitors. Fungal cells are homogenized and lysed with the Lysis Buffer containing chaotropic salts, detergents and denaturing agents. The lysate is cleared by centrifugation/ filtration, this step removes the cell debris, polysaccharides and other contaminants. The contaminants are washed away using different buffers during the washing steps. DNA is eluted with a low salt Elution Buffer which is ready for further downstream applications.

#### Unique Features: >

- Silica membrane technology
- Yield from 2-5 μgs DNA
- DNA obtained can be directly used for sequencing, PCR, restriction digestion etc.
- Lysis Buffer for the maximum yield from variety of Fungal samples.

#### Kit Contents:

- RNase A (Lyophilized)
- Proteinase K
- Lysis Buffer
- Wash Buffer I (W1) (concentrate)
- RT Wash Buffer II (W2) (concentrate)
- ◆ · Elution Buffer (EB)
- ◆ GeneiPure™ DNA Spin Columns
- Collection Tubes (2 ml)

#### References: >

- https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/fungus-isolation
- hen L, Fang Y, Zhu T, Gu Q, Zhu W (2008). "Gentisyl alcohol derivatives from the marine-derived fungus Penicillium terrestre".

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2117300021730	KT-300	GeneiPure™ Fungal DNA Purification Kit, 20 preps

#### gDNA Purification Kit - Cotton leaves, Seed and Lint

#### Description:

Several methods are available for extracting plant DNA. The protocol of isolating DNA using CTAB (Cetyltrimethylammonium ammonium bromide), a nonionic detergent, was initially used in bacteria (Jones, 1963) & later modified to obtain DNA from plants (Murray & Thompson 1980). Alternatively, SDS (Sodium Dodecyl Sulphate) can be used to lyse of plant cells. The major difficulty in obtaining pure DNA samples from plants is the high concentration of polyphenols, polysaccharides, terpenols and other substances. The CTAB method is one of the most widely used methods of DNA extraction and ensures high yields of pure DNA since it complexes with nucleic acids. For Rapid and simple purification of plant DNA, spin column technology has been employed. The gDNA Purification Kit - Cotton Leaves. Seed & Lint can be used for cotton plant samples containing different metabolites like polyphenols, polysaccharides or acidic components. Lysis Buffers have been optimized for processing cotton leaves, seed and lint Optimized Wash Buffers completely remove proteins, RNA and other PCR inhibitors. Tissue is homogenized and lysed with the Lysis Buffer containing chaotropic salts, detergents and denaturing agents. The lysate is cleared by centrifugation/ filtration this step removes the cell debris, polysaccharides and other contaminants. The supernatant is mixed with the Binding Buffer in order to facilitate optimum binding onto the GeneiPure TM Column. The contaminants are washed away using different buffers during the washing steps. DNA is eluted with a low salt Elution Buffer which is ready for further downstream applications.

#### GeNei Th

#### **Unique Features:**

- Silica membrane technology
- High yield from 5 μg 30 μg DNA
- DNA obtained can be directly used for cloning, PCR, restriction digestion etc.
- Filter column supplied helps in clearing the lysate before loading onto the GeneiPure TM Column.

#### Kit Contents:

- RNase A (Lyophilized)
- Lysis Buffer
- Precipitation Buffer
- Binding Buffer (Conc)
- Wash Buffer I (Conc)
- Wash Buffer II (Conc)
- Elution Buffer
- GeneiPure™ Columns
- Filter column
- Collection Tubes

#### Storage:Room temperature

#### Applications: >

- DNA Extraction from Plant tissues
- Purified DNA can be used in downstream applications like PCR, RAPD, Southern Blotting and other enzymatic reaction.

#### References:

- Neal Stewart, Jr., and Laura E. Via. A Rapid CTAB DNA Isolation technique Useful for RAPD Fingerprinting and Other PCR Applications. BioTechniques 1993 article Vol. 14(5):748-749.
- Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11-15.
- Doyle, J.J. and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue.
   Focus 12:13-15
- Jones A.S. and R.T. Walker, 1963, Isolation and analysis of the deoxyribonucleic acid of Mycoplasmamycoides var. Capri. Nature, 180, pp. 588-589.
- Mace ES, Buhariwalla HK, and Crouch JH (2003) A high-throughput DNA extraction protocol for tropical molecular breeding programs. Plant Mol Biol Rep 21: 459a-459h
- Murray M.G. and W.F. Thompson, 1980, Rapid isolation of high molecular weight plant DNA. Nucleic Acid Research, 8 (19), pp. 4321-4325.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2113050021730	KT305	gDNA Purification Kit - Cotton leaves, Seed and Lint, 20 preps

#### GeneiPureID<sup>™</sup> DNA Isolation Kits

#### GeneiPureID™ DNA Isolation Kit -Dried Blood

#### Description:

The ability to prepare and isolate pure nucleic acids from a variety of sources is an important step in many molecular biology protocols. The extraction of nucleic acids from biological samples is being practiced for example, in genetic engineering and construction of DNA probes. Various techniques have been employed for nucleic acid isolation. These techniques are widely used on a laboratory scale, but require technical skills and are difficult to reproduce. Forensic science demands the efficient purification of small amounts of DNA from a wide range of low-volume and trace samples. Blood, dried or fresh is a very important evidence in most of the forensic related cases. The presence of blood can associate a suspect to a victim or a suspect/victim to a crime scene. The type of bloodstains and dried blood recovered from a crime spot has added value as evidence in a criminal investigation. The physical appearance, staining for confirmation of human blood and DNA isolation are few of the methods used for further analysis of these blood samples.

The conventional nucleic acid isolation techniques have the following drawbacks:

- the starting amount of sample
- sample specificity for a kit
- yield of the DNA.

This kit requires minimum amount of sample for extraction of DNA from Dried Blood derived from crime scenes or subjects. The basic steps of DNA isolation includes: a) Sample collection b) Sample Lysis c) Binding of DNA to the column and Washes d) Elution

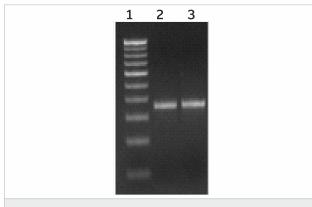
#### Unique Features: >

- Silica membrane technology
- Minimal amount of sample can be used
- Purified DNA obtained can be directly used for PCR.

#### Kit Contents:

- Lysis Buffer I Dried Blood
- ◆ · RT Lysis Buffer II Dried Blood
- Wash Buffer Dried Blood (Concentrate)
- Elution Buffer Dried Blood
- GeneiPureID Columns
- Collection Tubes

Storage: Room Temperature



**Fig 1:** PCR Amplification of 350 bp product of Human genomic DNA isolated from dried blood using this kit with cyt-b primers.

- **Lane 1**  $StepUp^{TM}$  100 Kb DNA Ladder
- Lane 2 PCR product of Dried Blood DNA from Paper
- Lane 3 PCR product of Dried Blood DNA from Cloth

#### Applications: >

- DNA isolated from forensic samples may be too scanty to visualize on an agarose gel. Hence it is amplified using PCR for detection.
- For screening and testing.

#### References: >

- Albarino CG. V. Romanowski. Phenol extraction revisited: a rapid method for the isolation and preservation of human genomic DNA from whole blood. Mol Cell Probes 1994;8:423-7.
- Parzer S, Mannhalter C. A rapid method for the isolation of genomic DNA from citrated whole blood. The Biochemical journal 1991;273(Pt 1):229-31 from citrated whole blood. Biochem J 273Pt 11991:229-31 4.
- Pochi R, Genomic DNA. Subbarayan, Malancha( et al) Isolation of from Human Whole Blood. BioTechniques December 2002;33 SRC GoogleScholar:1231-4.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2117900021730	KT179	GeneiPureID™ DNA Isolation Kit -Dried Blood, 10 preps
2117900031730	KT179L	GeneiPureID™ DNA Isolation Kit -Dried Blood, 50 preps

#### GeneiPureID™ DNA Isolation Kit for semen

#### Description:

The ability to prepare and isolate pure nucleic acids from a variety of sources is an important step in many molecular biology protocols. The extraction of nucleic acids from biological samples is being practiced, for example, in genetic engineering and in construction of DNA probes. Various techniques have been employed for nucleic acid isolation and are widely used on a laboratory scale but require technical skills and are difficult to reproduce. Forensic science demands the efficient isolation of small amounts of DNA from a wide range of low-volume and trace samples. Recovery of high molecular weight genomic DNA

The GeneiPureIDTM DNA Isolation Kit- Semen provides a rapid, organic free method for the isolation of genomic DNA from fresh, frozen, and dry semen sample including semen spots found on filter paper, floor, cloth etc. The optimized protocol results in high quality and quantity of DNA suitable for all downstream applications. The kit allows single or multiple processing of samples simultaneously, does not require any Phenol/chloroform extractions and is less time consuming. Semen samples after proper washing are lysed using appropriate lysis buffer with DTT. Following lysis, the inhibitors and other contaminants are removed in the presence of high salt concentration and then loaded on to silica membrane column. After a brief centrifugation, DNA is selectively bound to the membrane while the contaminants pass through. Inhibitors are completely removed by effective washes and pure nucleic acid is eluted in minimal volume of elution buffer.

#### Unique Features:

- ◆ Recovery of genomic DNA upto10-15µg from 100ul of fresh semen (Refer Fig 1).
- Quick and Simple.
- No RNA Contamination.
- No Phenol/Chloroform Extraction.
- Good quality DNA for downstream applications.

#### **Kit Contents:**

- Lysis Buffer Semen
- Binding Buffer Semen
- Wash Buffer I Semen
- Wash Buffer II Semen
- Elution Buffer Semen
- GeneiPureIDTM Columns
- 2 ml Collection Tubes
- Instruction Manual

Storage: Room Temperature

#### Applications:

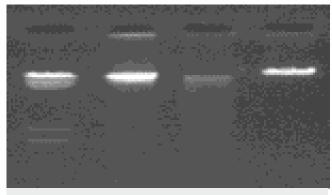
The purified genomic DNA is ready for use in downstream applications like:

- Restriction Analysis
- PCR
- Southern Blotting
- Human forensic
- Haploid DNA study
- Diagnostics

М

2

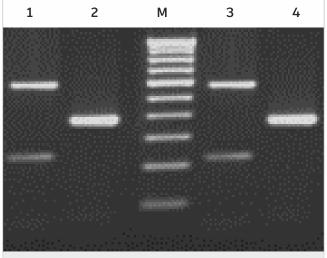
3



Analysis of DNA isolated from fresh and dry semen using GeneiPure IDTM DNA Isolation Kit on agarose gel.

- Lane M
- YDNA/HindIII marker
- Lane 2
- Fresh semen DNA
- - Y DNA (undigested)

- Fillter paper blotted semen DNA Lane3 Lane 4



Analysis of PCR amplicons of DNA from fresh and dry semen using Alu PCR of Plat gene and Cyt-b primers

- Lane 1 Alu PCR of Plat gene from fresh semen
- Lane 2 Cyt-b from fresh semen
- Lane M 100 bp ladder
- Lane 3 Alu PCR of Plat gene from dry semen
- Lane 4 Cyt-b from dry semen

#### References:

- Shiurba R, Nandi S. Isolation and characterization of germ line DNA from mouse sperm. Proc. Natl. Acad. Sci. USA. 1979;76:3947-3951
- Griffin J. Methods of sperm DNA extraction for genetic and epigenetic studies. Methods Mol. Biol. 2013;927:379-384.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2118000021730	KT180	GeneiPureID™ DNA Isolation Kit-Semen, 10 preps

#### GeneiPureID™ DNA **Isolation Kit-Bone**

#### Description:

The ability to prepare and isolate pure nucleic acids from a variety of sources is an important step in many molecular biology protocols. The extraction of nucleic acids from biological samples is being practiced, for example, in genetic engineering and in construction of DNA probes. Various techniques have been employed for nucleic acid isolation and are widely used on a laboratory scale, but require technical skills and are difficult to reproduce. Forensic science demands the efficient isolation of small amounts of DNA from a wide range of low-volume and trace samples.

The GeneiPureID DNA Isolation Kit - Bone provides a rapid and simple method for the isolation of ready to use genomic DNA from fresh, cooked and ancient bones. The protocol results in high quality and quantity of DNA, which can be used for PCR and other downstream applications for various studies. Bone samples after complete decalcification are lysed using lysis buffer with Proteinase K. Following lysis, inhibitors and other contaminants are removed in presence of high salt concentration and then loaded on to a silica membrane column. After a brief centrifugation, DNA is selectively bound to the membrane while the contaminants pass through. Inhibitors are completely removed by effective washes and pure nucleic acid is eluted in minimal volume of elution buffer.

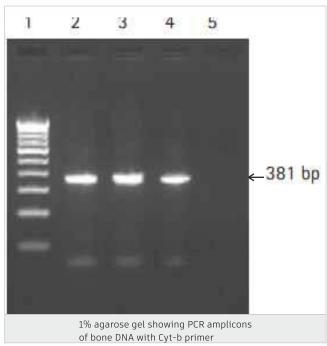
#### Unique Features:

- Recovery of genomic DNA upto10-15µg from 250 mg of fresh bone and 100-900ng from cooked and ancient bone.
- Quick and Simple.
- No RNA Contamination.
- No Phenol/ Chloroform Extraction.
- Good quality DNA for downstream applications.

#### Kit Contents:

- ◆ Lysis Buffer I- Bone
- Lysis Buffer II Bone
- Binding Buffer Bone
- Wash Buffer I Bone
- Wash Buffer II Bone
- Elution Buffer-Bone
- GeneiPureID™ Columns
- Collection Tubes
- ◆ EDTA
- Instruction Manual

Storage: Room Temperature.



Lane 1 - StepUp™ 100 bp DNA marker

Lane 2 - Fresh Bone

Lane 3 - Cooked Bone

Lane 4 - Ancient Bone

Lane 5 - Negative-PCR

#### Applications: >

The pure genomic DNA is ready for use in downstream application like:

- Restriction analysis
- PCR
- Phylogenetic studies
- Population genetics
- Wild life and human forensics

#### References:

- High efficiency DNA extraction from bone by total demineralization Forensic Sci. Int. Genet.
- Comparison of three methods of DNA extraction from human bones with different degrees of degradation -Int. J. Legal Med

Cat. No	PI No.	Product Description
2118100021730	KT181	GeneiPureID™ DNA Isolation Kit-Bone, 10 preps
2118100031730	KT181L	GeneiPureID™ DNA Isolation Kit-Bone, 50 preps



# GeneiPureID™ DNA Isolation Kit-Saliva

#### Description:

The ability to prepare and isolate pure nucleic acids from a variety of sources is an important step in many molecular biology protocols. The extraction of nucleic acids from biological samples is being practiced, for example, in genetic engineering and in construction of DNA probes. Various techniques have been employed for nucleic acid isolation and are widely used on a laboratory scale, but require technical skills and are difficult to reproduce. Forensic science demands the efficient isolation of small amounts of DNA from a wide range of lowvolume and trace samples. Saliva is a very significant sample in many forensic cases e.g. traces of Saliva on the dress, envelope, crime spot, buccal swabs, cigarette butts stained with saliva etc. Saliva analysis is part of a routine autopsy examination. It is analyzed by different methods such as Gas/liquid chromatography, DNA isolation etc.

The conventional nucleic acid isolation techniques have the drawbacks of

- the starting amount of sample
- sample specificity of a kit,
- yield of DNA.

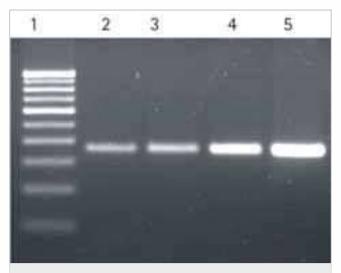
#### Unique Features:

- Silica membrane technology
- Minimal amount of sample can be used
- Purified DNA obtained can be directly used for PCR with gene specific primers.

#### Kit Contents:

- Lysis Buffer I Saliva
- Lysis buffer II Saliva
- Wash Buffer (concentrate) Saliva
- Elution Buffer Saliva
- GeneiPureID™Columns
- ◆ · 2 ml Collection Tubes
- Instruction Manual

Storage: Room Temperature



Amplification product of human genomic DNA isolated from different starting amount of saliva using cyt-b primer

**Lane1** - StepUp<sup>™</sup> 100 bp DNA ladder

Lane 2 - 5 µl of Saliva

Lane 3 - 50 µl of Saliva

Lane 4 - 200 µl of Saliva

Lane 5 - 2 ml of Saliva

#### Applications:

The pure genomic DNA is ready for use in downstream application like:

- Restriction analysis
- PCR
- Phylogenetic studies

#### References:

- Comparison of different collection procedures and two methods for DNA isolation from saliva Jaroslava Durdiakov á 1,2, Nat á lia Kamodyov á 1, Daniela Ostatní kov á 2, Barbora Vlkov á 1 and Peter Celec 1,3
- Herraez DL, Stoneking M. High fractions of exogenous DNA in human buccal samples reduce the quality of large-scale genotyping. Anal Biochem 2008;383:329 – 31
- Nedel F, Conde MC, Oliveira IO, Tarquinio SB, Demarco FF. Comparison between DNA obtained from buccal cells of the upper and lower gutter area. Braz Dent J 2009;20:275 – 8

Cat. No	PI No.	Product Description
2118400021730	KT184	GeneiPureID™ DNA Isolation Kit-Saliva, 10 preps

#### GeneiPureID™ DNA Isolation Kit-Skin

#### Description: >

Skin is the outer covering of the body. In humans, it is the largest organ of the integumentary system made up of multiple layers of mesodermal tissues that guards the underlying muscles, bones, ligaments and internal organs. Skin plays a key role in protecting the body against pathogens and excessive water loss. The ability to prepare and isolate pure nucleic acids from a variety of sources is an important step in many molecular biology protocols. The extraction of nucleic acids from biological samples is being practiced, for example, in genetic engineering and in construction of DNA probes. Various techniques have been employed for nucleic acid isolation and are widely used on a laboratory scale, but require technical skills and are difficult to reproduce. Forensic science demands the efficient isolation of small amounts of DNA from a wide range of low-volume and trace samples. The GeneiPureID DNA Isolation Kit - Skin provides a rapid, simple organic free method for the isolation of ready to use genomic DNA from skin. The protocol results in high quality of DNA from fresh / dry and burnt / decayed skin which can be used for PCR and other downstream applications.

#### Unique Features:

- Quick and Simple.
- No RNA Contamination.
- No Phenol/Chloroform Extraction.
- Good quality DNA for downstream applications.

#### Kit Contents:

- Proteinase K
- Lysis Buffer
- Binding Buffer
- Wash Buffer I
- Wash Buffer II
- Elution Buffer
- ◆ GeneiPureID™ Spin Column
- Collection Tubes

Storage: Room Temperature

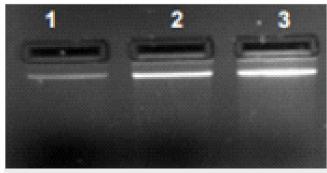


Fig 1: Analysis of high molecular weight DNA isolated from 25 mg goat skin using GeneiPureID™ DNA Isolation Kit – Skin

Lane 1 - 2 µl Eluted DNA | Lane 2-5 µl Eluted DNA | Lane 3-8 µl Eluted DNA

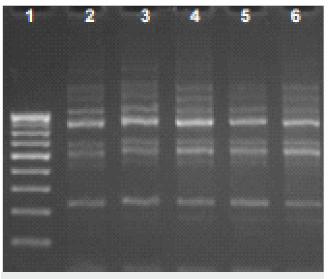


Fig 2: RAPD profile of DNA isolated from goat skin (25 mg) using 18 mer primer

Lane 1 - StepUp<sup>™</sup> 100bp DNA Ladder Lane 4&5 - DNA from decayed skin
Lane 2&3 - DNA from fresh skin Lane 6 - DNA from bumt skin

#### Applications:

- Phylogenetic studies
- Wild life and human forensics
- Clinical study

#### References:

- Effects of sampling strategy and DNA extraction on human skin microbiome investigations-Rie Dybboe Bjerre, Luisa Warchavchik Hugerth, Fredrik Boulund, Maike Seifert, Jeanne Duus Johansen & Lars Engstrand
- Hugerth, L. W. et al. A comprehensive automated pipeline for human microbiome sampling, 16S rRNA gene sequencing and bioinformatics processing. bioRxiv, 286526.

Cat. No	PI No.	Product Description
2121000011730	KT210	GeneiPureID™ DNA Isolation Kit-Skin, 10 preps



#### **Products for RNA**

#### **Total RNA Isolation**

#### Other Related Products

#### **Solution Based Kits:**

- TRISol
- ▶ Plant RNA Isolation Kit
- RaFlex<sup>™</sup> Total RNA Isolation Kit (For Plants)
- RaFlex<sup>™</sup> Total RNA Isolation Kit (For Blood, Animal Cells and Tissues, Bacteria)

#### Column Based Kits: GeneiPure™ Total RNA Isolation Kits for

- Cells and Tissue
- Blood
- Plants
- Bacteria
- Yeast

#### GenPro™3-in-1 Isolation Kits:

- Cells and Tissue
- Blood
- Plants
- Bacteria

#### cDNA Synthesis:

- DNA Direct Kit
- One Step M-MuLV RT-PCR Kit
- M-MuLV RT-PCR Kit
- RT III RT-PCR Kit
- Recombinant RNase Inhibitor

#### **RNA Electrophoresis:**

- > 5X RNA Gel Loading Buffer
- ▶ EGK 7

#### Other Fine Chemicals for RNA Work:

- DNase I (RNase Free)
- Water (DNase, RNase Free)
- Saturated Phenol (Water)
- Guanidine Thiocyanate
- > 0.5M EDTA (DNase, RNase Free)
- ▶ 10X TE (DNase, RNase Free)
- M Tris-Cl (pH 8.0)
- Lymphocyte Separating Solution
- ▶ 50X Denhardt's Reagent
- RNA Integra
- Sodium Acetate 3M (DNase, RNase Free)
- 3M Potassium Acetate (pH 5.5) (DNase, RNase Free)

GENOMICS

# GeNei

# Total RNA Isolation Selection Guide

n 2	Product Name	Starting material	Yeld	Highlights	Process Speed	A A	NT-PCI
#	Rattes "Total Rotal Societion Vit (For Plants)	Plant tissue	130-150µg from 100mg Amabisleaf     80-90µg from 100mg Picrorhizaroot     15-20µg from 100mg almond back     15-20µg from 100mg almond back     40-50µg from 100mg tomato stem     80-100µg from 100mg of lemongrass	Solution based     Universal bit for all types of tissues     Highly efficient evenfor difficult plant tissues with high quantity of pigments and secondary metabolites.     Supplied with RNA integra (RNA storagebuffer).	:		¥
~	Rather** Total RNA isolation kit (ForBlood, Animalcells and tissum, bacteria)	Animal cells and trissue, lifecod, Bactoria	130 150 yg from 2x30" Hela cells     200 220 yg from 190 mg mouneliver     90 100 yg from 1ml of Overnight grown culture of gramsvebacteria     15-2 yg from 0.1ml of blood	Solution based     Maximum yield     No RBC lysis required for Blood     Supplied with RNA Integra (RNA storage buffer).	:	:	E 453
10	TRISolin	Animal cells and thous, bacteria	70-100µg from 2x10" Hela cells     70-100µg from 100mg mouseliver/brain/heart	Single reagent for isolation of RNA DNA and protein	:	1	ş
-0	Plant Total RNA Holation kit	Plant bissues	80 120 <sub>kg</sub> from 100mg of green gram sprouts	Solution based  • Lithium chloride based method	*		ē
4	Genethure* Total RNA Isotation kit cells and Usaue	Animal cells and fixures	40-60µg from2x10* Hela cells     50-60µg from 10mg of mouse livertissue     7-10µg from 10mg Mouse hearttissue		•		Ē
	General Total RNA Isolation kit Bacteria	Bacteria	15:50ug from Lmi overnight culture			ŧ	166
1 5	Process speed is the time taken complete the extended	committees the sense	Annual Control of the	20 mm			

saturated 00 v....

\*\*\* 60 to 90minutes

· 90 to 120 minutes

\*>120 minutes

\*\*\*151018 \*\*\*\* \$ 10 2

The column based kits are convenient for high throughputs creening The solution based kits are convenient in scaling up.

RNA purified using all the above. Wits perform in Reverse Transcription reaction



#### RaFlex Total RNA **Isolation Kit** (for Plants)

#### Description:

Isolation of intact RNA is a pre-requisite for the analysis of gene expression. One of the most important criterion in the isolation of Total RNA is to prevent its degradation during the isolation procedure. RNA is generally isolated by using strong denaturants like guanidine thiocyanate and phenolchloroform with a reducing agent, mercaptoethanol, to inhibit the RNase activity. Most methods do not yield DNA free RNA and would require the samples to be further treated with DNase I. Other methods include the use of Lithium chloride to selectively precipitate RNA. Successful isolation of RNA depends on the suppression of endogenous RNAses during cell / tissue lysis and avoiding contamination with exogenous RNAses during the isolation procedure.

RaFlex<sup>™</sup> Total RNA Isolation Kit is a convenient. rapid and efficient way of extracting RNA from plant tissue. RaFlex™ Total RNA isolation kit is a solution based method for the isolation of RNA. It is optimized for high yield, intact total RNA extraction from a wide variety of tissues. This procedure involves tissue homogenisation, centrifugation and precipitation of RNA. The precipitated RNA pellet is stabilized and stored in RNAIntegra™, a unique storage buffer safeguarding the integrity of RNA much longer than when stored in nuclease free water or Tris buffer.

#### Unique Features:

- Rapid: Total time taken per isolation is about 1-2
- Flexible: Mini, Midi and Maxi preps can be done using the same kit.
- Consistent and highly reproducible results.
- Tested and proved in 39 plant tissues across various plant species belonging to diverse genera and those rich in various types of secondary metabolites:

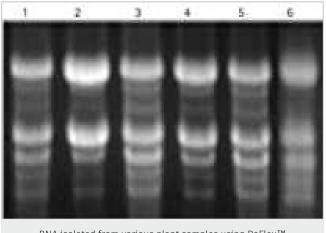
- Works exceptionally well even with Jatropha, Witahnia (root) and Cotton, wherein the performance of most other RNA isolation systems are not satisfactory.
- RNAIntegra<sup>™</sup> a unique buffer for suspending the purified RNA is provided in the kit that enhances the shelf life of the purified RNA.

#### Kit Contents:

- RNAIntegra<sup>™</sup>
- RaFlex™ Solution I
- RaFlex™ Solution II
- RaFlex™ Solution III

#### Applications:

 RNA obtained can be used for several downstream applications such as cDNA synthesis, Northern blotting, RT-PCR, mRNA purification etc



RNA isolated from various plant samples using RaFlex™

Lane 1 Rheum Leaf

Lane 2 - Arabidopsis Leaf

Lane 3 -Tea Leaf

Lane 4 - Caragana Buds

Lane 5 - Stevia Leaf

Lane 6 - Arnebia Leaf

#### References:

- Chomczynski P and Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenolchloroform extraction. Anal Biochem, 162: 156-159.
- Chomczynski P (1993) A reagent for the Single-step simultaneous isolation of RNA, DNA and Proteins from cell and tissue samples BioTechniques. 15: 532-537...
- Mackey K and Chomczynski P (1996) Long term stability of RNA isolation reagents. J NIH Res, 8: 72-78. 4. Chomczynski P and Mackey K (1995) Substitution of chloroform with Bromochloropropane in the singlestep method of RNA isolation Anal Biochem, 225: 163-164.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2115100021730	KT151L	RaFlex Total RNA Isolation Kit (for Plants), 50 preps

RaFlex<sup>TM</sup> Total RNA
Isolation Kit (for Bacteria,
Blood, Animal Cells & Tissues)

#### Description: >

Isolation of intact RNA is a pre-requisite for the analysis of gene expression. One of the most important criterion in the isolation of Total RNA is to prevent its degradation during the isolation procedure. RNA is generally isolated by using strong denaturants like quanidine thiocyanate and phenolchloroform with a reducing agent, mercaptoethanol, to inhibit the RNase activity. Most methods do not yield DNA free RNA and would require the samples to be further treated with DNase I. Other methods include the use of Lithium chloride to selectively precipitate RNA. Successful isolation of RNA depends on the suppression of endogenous RNAses during cell / tissue lysis and avoiding contamination with exogenous RNAses during the isolation procedure.

RaFlex<sup>™</sup> Total RNA Isolation Kit is a convenient, rapid and efficient way of extracting RNA from plant tissue. RaFlex<sup>™</sup> Total RNA isolation kit is a solution based method for the isolation of RNA. It is optimized for high yield, intact total RNA extraction from a wide variety of tissues. This procedure involves tissue homogenisation, centrifugation and precipitation of RNA. The precipitated RNA pellet is stabilized and stored in RNAIntegra<sup>™</sup>, a unique storage buffer safeguarding the integrity of RNA much longer than when stored in nuclease free water or Tris buffer.

#### **Unique Features:**

- Rapid: Total time taken per isolation is about 1-2 hours.
- Flexible: Mini, Midi and Maxi preps can be done using the same kit.
- Consistent and highly reproducible results.
- Tested for various cells and tissue samples.
- Erythrocyte lysis step not required for blood samples.
- RNAintegra™ a unique buffer for re-suspending the purified RNA, provided in the kit, enhances the shelf life of the purified RNA.

#### Kit Contents:

- RNAIntegra™
- RaFlex™ Solution I
- RaFlex™ Solution II

Storage: Room Temperature

#### Applications: >

 RNA obtained is can be used for several downstream applications such as cDNA synthesis, Northern blotting, RT-PCR, mRNA purification etc

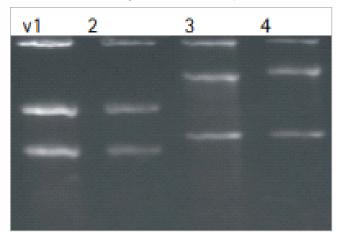


Fig 1: RNA isolated from various tissues using RaFlex™

Lane 1 - Gram Positive Bacteria
Lane 2 - Gram Negative Bacteria
Lane 3 - Heart Tissue
Lane 4 - Hela Cells

#### References:

 Chomczynski P and Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenolchloroform extraction. Anal Biochem, 162: 156-159.



- Chomczynski P (1993) A reagent for the Single-step simultaneous isolation of RNA, DNA and Proteins from cell and tissue samples BioTechniques. 15: 532-537..
- Mackey K and Chomczynski P (1996) Long term stability of RNA isolation reagents. J NIH Res, 8: 72-78. 4. Chomczynski P and Mackey K (1995) Substitution of chloroform with Bromochloropropane in the singlestep method of RNA isolation Anal Biochem. 225: 163-164.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2115100031730	KT151A	RaFlex Total RNA Isolation Kit (Animal Cells & Tissues,Blood, Bacteria), 50 preps

#### Plant RNA isolation kit

#### Description: >

Plant RNA isolation kit produces high yields of intact total RNA from a variety of plant tissues. The protocol involves tissue disruption by SDS followed by acid-phenol-chloroform extraction and selective precipitation of RNA. RNA obtained is good for several downstream applications such as cDNA synthesis, mRNA purification, RT-PCR etc. The quality and quantity of RNA extracts were tested by measurement of A260/A280 ratio and subsequently by electrophoresis on formaldehyde agarose gel.

Storage: 4°C

#### Unique Features:

- Reproducible, reliable, economical and rapid method of RNA isolation
- Higher yields of RNA obtained when compared to traditional methods
- Isolated RNA suitable for RT-PCR, cDNA synthesis, mRNA purification etc.
- ◆ Ratio of A<sub>260</sub>/A<sub>280</sub> is greater than 1.7

#### Applications: >

 RNA obtained can be used for several downstream applications such as cDNA synthesis, Northern blotting, RT-PCR, mRNA purification etc

Biological Source	Yield total RNA(µg)
Plant (500 mg leaves)	
Tomato Leaf	475-525
<ul> <li>Tomato Green Fruit</li> </ul>	80-90
<ul> <li>Withania Leaf</li> </ul>	600-650
Banana	55-60
• Tea	50-60
1 2 3 4 5	6
	Lane 1 : Tomato
	Lane 2 : Rice
	Lane 3 : Tobacco
+	Lane 4 : Tea
THE RESIDENCE	Lane 5 : Banana
	Lane 6 : Cotton

Total RNA isolated from different plant species.

#### References:

 Maniatis, T. Fritsch, E.F. & Sambrook, J. (1982), Molecular cloning: A laboratory manual p. 202, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2109800011730	KT98	GeNei™ Plant RNA Isolation Kit, 10 preps

#### GeneiPure™ Total RNA Isolation

GeneiPure™ Total RNA Isolation Kit-Cells and Tissues

#### Description:

Isolation of intact RNA is a pre-requisite for the analysis of gene expression. One of the most important criterion in the isolation of total RNA is to prevent its degradation during the isolation procedure. Description: The GeneiPure Total RNA isolation kit provides a fast and simple method of isolating total RNA from animal cells and tissues. The procedure represents a well-established spin column technology for RNA purification. Cell and

Tissue samples are first lysed and homogenized by incubating in a solution containing large volumes of chaotropic ions. The Lysis Buffer immediately inactivates RNases and creates an appropriate binding condition, which favours adsorption of RNA to the silica membrane. The washing steps with two different wash buffers remove salts, metabolites and macromolecular cellular components. Pure RNA is eluted under low ionic strength conditions with RNase-free water.

#### Unique Features:

- Spin column technology.
- Quick and simple procedure- recovery of pure and intact RNA in less than 30 minutes.
- No phenol/chloroform extraction or ethanol precipitation.
- Compatible with wide range of cell lines and tissues.
- ◆ Highly pure RNA is obtained with A260/A280 of 2.0-2.1
- Minimal or no genomic DNA contamination.
- RNA obtained is of high quality and can be directly used for all downstream applications.

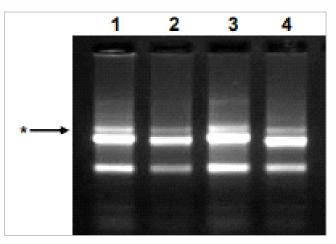
#### Kit Contents:

- Proteinase K
- Lysis BufferCells and Tissue
- Wash Buffer ICells & Tissues (concentrate)
- Wash Buffer IICells & Tissues (concentrate)
- Elution Buffer
- Filtration Columns
- GeneiPure RNA Columns
- Collection Tubes

#### Applications:

The purified RNA is ready for use in downstream applications such as:

- RT-PCR and Real-Time RT-PCR
- cDNA Synthesis
- Northern Blot
- RNase/S1 Nuclease Protection
- Primer Extension
- Microarrays



Analysis of Total RNA isolated from 1x106 cells from different mammalian cell lines using GeneiPure Total RNA Isolation Kit- Cells and Tissues, on 1% Agarose Gel.

Lane 1 - Total RNA isolated from HeLa cells

Lane 2 - Total RNA isolated from MCF-7 cells

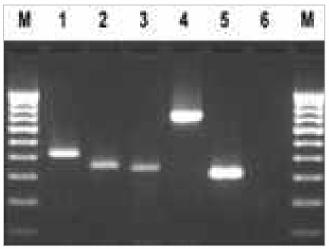
Lane 3 - Total RNA isolated from COS-7 cells

Lane 4 - Total RNA isolated from NIH3T3 cells

Yield of total RNA obtained from different Mouse tissues using GeneiPure Total RNA Isolation Kit-

Tissue (10 mg)	Yield of Total RNA (µg)
Liver	50-60
Kidney	20-30
Pancreas	30-35
Spleen	25-30
Heart	7-10
Skeletal muscle	12-15
Brain	7-10
Lung	10-15
Ovary	6-8
Testis	5-8

Analysis of Total RNA isolated from 1x106 cells from different mammalian cell lines using GeneiPure Total RNA Isolation Kit- Cells and Tissues, on 1% Agarose Gel.



Analysis of RT-PCR of different gene fragments amplified from total RNA isolated from HeLa cells using GeneiPureÔ Total RNA Isolation Kit- Cells and Tissues, on a 2% agarose gel.

#### GeNei Th

Lane M - StepUp<sup>™</sup> 100 bp DNA Ladder

Lane 1 - 412 bp fragment of p53 gene

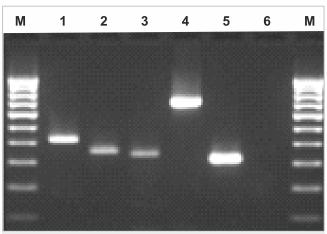
Lane 2 - 353 bp fragment of BRCA2

 $\textbf{Lane 3 -} \quad 339\,bp\,fragment\,of\,c\text{-myc\,gene}$ 

Lane 4 - 716 bp fragment of b-action gene

Lane5 - 323 bp fragment of GAPDH gene

Lane 6 - Negative RT-PCR



Analysis of RT-PCR amplified 323 bp GAPDH gene fragment from varying number of HeLa cells, on a 2% agarose gel.

**Lane M** - StepUp<sup>™</sup> 100 bp DNA Ladder

**Lane 1** - RT-PCR amplification of GAPDH gene from 10<sup>3</sup> cells

Lane 2 - RT-PCR amplification of GAPDH gene from 10<sup>4</sup> cells

Lane 3 - RT-PCR amplification of GAPDH gene from 10⁵ cells

Lane 4 - Negative RT-PCR

#### References:

- Mackey K and Chomczynski P (1996) Long term stability of RNA isolation reagents. J NIH Res, 8: 72-78. 4. Chomczynski P and Mackey K (1995) Substitution of chloroform with Bromochloropropane in the singlestep method of RNA isolation Anal Biochem. 225: 163-164.
- Maniatis, T. Fritsch, E.F. & Sambrook, J. (1982), Molecular cloning: A laboratory manual p. 202, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

#### **Ordering Information**

Cat. No	PI No.	Product Description
2117100021730	KT171	GeneiPure™ Total RNA Isolation Kit- Cells and Tissues, 20 preps

# GeneiPure™ Total RNA Isolation Kit -Bacteria

#### Description:

GeneiPure™ Total RNA Isolation Kit provides a fast and simple method of isolating total RNA from various biological samples like animal cells and tissues, blood (white blood cells), yeast, bacteria and plants. The procedure represents a well established silica membrane technology with a spin column format for rapid, bind-wash and elute method to prepare high quality total RNA. Biological samples are first lysed and homogenized by using a single lysis buffer containing chaotropic ions which immediately inactivate RNases and create an appropriate binding condition, which favors adsorption of RNA to the silica membrane. Following different washes to remove salts metabolites and macromolecular cellular components pure RNA is eluted under low ionic strength conditions with RNase -free water.

#### Unique Features: >

- High recovery of pure and intact RNA
- Quick and Simple.
- No DNA Contamination.
- No Phenol/Chloroform Extraction. Complete column based purification

#### Kit Contents:

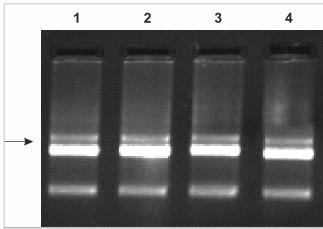
- Proteinase K
- Lysozyme
- Lysis Buffer Bacteria
- Wash Buffer I Bacteria (concentrate)
- Wash buffer IIBacteria (concentrate)
- 1XTE Buffer
- Flution Buffer
- Filtration Columns
- ◆ GeneiPure RNA Columns
- Collection Tubes

#### Applications: >

 The purified RNA is ready for use in downstream applications such as: cDNA Synthesis RT-PCR and Real-Time PCR Northern Blot Microarray

Yield of total RNA obtained from 1 ml of bacterial strains using GeneiPure Total RNA Isolation KitBacteria

Bacterial species	Yield of Total (µg)
B. subtilis	15-25
Staphylococcus sp.	20-30
E. coli	30-50
Pseudomonas sp.	15-25



- \*Represents high molecular weight RNA
- Analysis of Total RNA from different Bacterial strains using GeneiPure Total RNA Isolation Kit-Bacteria on a 1% Agarose Gel.
- Lane 1 Total RNA isolated from B. subtilis
- Lane 2 Total RNA isolated from Staphylococcus sp.
- Lane 3 Total RNA isolated from E. coli
- Lane 4 Total RNA isolated from Pseudomonas sp.

#### References:

- Chomczynski P and Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem, 162:156-159.
- Chomczynski P (1993) A reagent for the Singlestep simultaneous isolation of RNA, DNA and Proteins from cell and tissue samples. BioTechniques, 15: 532-537.
- Mackey K and Chomczynski P (1996) Long term stability of RNA isolation reagents. J NIH Res, 8: 72-78. 4. Chomczynski P and Mackey K (1995) Substitution of chloroform with Bromochloropropane in the singlestep method of RNA isolation. Anal Biochem, 225:163-164

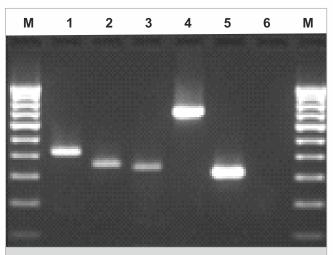
#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2117500021730	KT175	GeneiPure™ Total RNA Isolation Kit - Bacteria, 20 preps

#### MicroRNA Isolation Kit- Cells & tissues

#### Description: >

GeneiPure™ Total RNA Isolation Kit provides a fast and simple method of isolating total RNA from various biological samples like animal cells and tissues, blood (white blood cells), yeast, bacteria and plants. The procedure represents a well established silica membrane technology with a spin column format for rapid, bind-wash and elute method to prepare high quality total RNA. Biological samples are first lysed and homogenized by using a single lysis buffer containing chaotropic ions which immediately inactivate RNases and create an appropriate binding condition, which favors adsorption of RNA to the silica membrane. Following different washes to remove salts metabolites and macromolecular cellular components pure RNA is eluted under low ionic strength conditions with RNase-free water.



Analysis of RT-PCR amplified 323 bp GAPDH gene fragment from varying number of HeLa cells, on a 2% agarose gel.

**Lane M** - StepUp<sup>™</sup> 100 bp DNA Ladder

**Lane 1** - RT-PCR amplification of GAPDH gene from 10<sup>3</sup> cells

Lane 2 - RT-PCR amplification of GAPDH gene from 10<sup>4</sup> cells

Lane 3 - RT-PCR amplification of GAPDH gene from 10<sup>5</sup> cells

Lane 4 - Negative RT-PCR

- Mackey K and Chomczynski P (1996) Long term stability of RNA isolation reagents. J NIH Res, 8: 72-78. 4. Chomczynski P and Mackey K (1995) Substitution of chloroform with Bromochloropropane in the singlestep method of RNA isolation Anal Biochem. 225: 163-164.
- Maniatis, T. Fritsch, E.F. & Sambrook, J. (1982), Molecular cloning: A laboratory manual p. 202, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York



#### **Ordering Information**

Cat. No	PI No.	Product Description
2117100021730	KT171	GeneiPure™ Total RNA Isolation Kit- Cells and Tissues, 20 preps

# GeneiPure™ Total RNA Isolation Kit -Bacteria

#### Description:

GeneiPure™ Total RNA Isolation Kit provides a fast and simple method of isolating total RNA from various biological samples like animal cells and tissues, blood (white blood cells), yeast, bacteria and plants. The procedure represents a well established silica membrane technology with a spin column format for rapid, bind-wash and elute method to prepare high quality total RNA. Biological samples are first lysed and homogenized by using a single lysis buffer containing chaotropic ions which immediately inactivate RNases and create an appropriate binding condition, which favors adsorption of RNA to the silica membrane. Following different washes to remove salts metabolites and macromolecular cellular components pure RNA is eluted under low ionic strength conditions with RNase-free water.

#### Unique Features:

- High recovery of pure and intact RNA
- Quick and Simple.
- No DNA Contamination.
- No Phenol/Chloroform Extraction. Complete column based purification

#### Kit Contents:

- Proteinase K
- Lysozyme
- Lysis Buffer Bacteria
- Wash Buffer I Bacteria (concentrate)
- Wash buffer IIBacteria (concentrate)

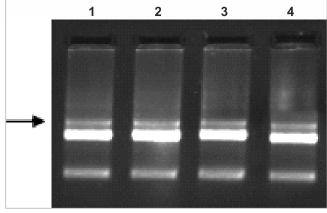
- 1X TE Buffer
- Elution Buffer
- Filtration Columns
- GeneiPure RNA Columns
- Collection Tubes

#### Applications: >

 The purified RNA is ready for use in downstream applications such as: cDNA Synthesis RT-PCR and Real-Time PCR Northern Blot Microarray

Yield of total RNA obtained from 1 ml of bacterial strains using GeneiPure Total RNA Isolation KitBacteria

Bacterial species	Yield of Total (µg)
B. subtilis	15-25
Staphylococcus sp.	20-30
E. coli	30-50
Pseudomonas sp.	15-25



\*Represents high molecular weight RNA

- Analysis of Total RNA from different Bacterial strains using GeneiPure Total RNA Isolation Kit- Bacteria on a 1% Agarose Gel.
- Lane 1 Total RNA isolated from B. subtilis
- Lane 2 Total RNA isolated from Staphylococcus sp.
- Lane 3 Total RNA isolated from E. coli
- Lane 4 Total RNA isolated from Pseudomonas sp.

#### References:

- Chomczynski P and Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem, 162: 156-159.
- Chomczynski P (1993) A reagent for the Singlestep simultaneous isolation of RNA, DNA and Proteins from cell and tissue samples. BioTechniques, 15: 532-537.
- Mackey K and Chomczynski P (1996) Long term stability of RNA isolation reagents. J NIH Res, 8: 72-78. 4. Chomczynski P and Mackey K (1995) Substitution of chloroform with Bromochloropropane in the single-step method of RNA isolation. Anal Biochem, 225: 163-164

#### MicroRNA Isolation Kit- Cells & tissues

#### Description:

MicroRNA (miRNA) are non-coding approximately 20-24 nucleotide small RNAs which are naturally expressed in animal cells and tissues. MicroRNAs are involved in various biological processes and are actively involved in cellular differentiation, signal transduction, protein degradation, response to biological stress and pathogen invasion. They also regulate the expression of various important genes.

MicroRNA Isolation Kit for Animal Cells and Tissues provides a simple, rapid and efficient column-based method for purifying and enriching pure and high-quality microRNA (miRNA) from a wide range of animal cells and tissues within 30 minutes. The formulation of the buffer is such that it will help to extract and selectively bind small RNA to the silica membrane in the presence of an organic solvent.

This kit helps in the isolation of enriched fraction of pure small RNA below 250bp including tRNA, 5S rRNA, 5.8S rRNA and regulatory RNA molecules such as microRNAs (miRNA).

#### Unique Features:

- Spin column technology.
- Rapid purification and enrichment of microRNA within 30 minutes
- Minimizes contamination with large RNA and DNA molecules
- Suitable for isolating small RNA
- · Ideal for miRNA, siRNA, snRNA analysis.
- Compatible with wide varieties of animal tissues and cell lines.

#### Kit Contents:

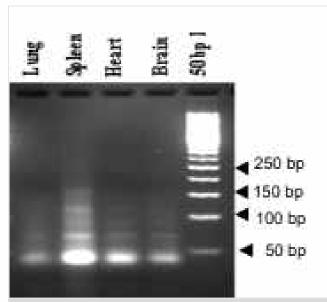
- ◆ Cells & Tissue miRNA Lysis Buffer
- Cells & Tissue miRNA Wash Buffer-I
- ◆ Cells & Tissue miRNA Wash Buffer-II
- Filtration Column
- Enrichment Column

- Collection Tubes
- Elution Buffer

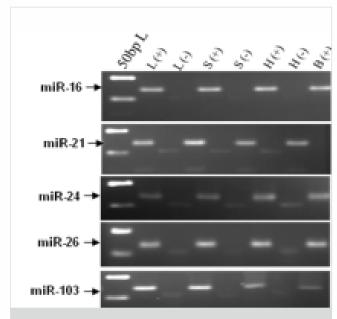
#### Applications:

The purified small RNA are suitable for applications like:

- Relative Quantification with Real Time PCR
- Microarray Hybridization Assay
- Reverse Transcriptase PCR



Analysis of miRNA isolated fro, different mouse tissues using miRNA isolation Kit - Cells and Tissues on a 1.5 % Agarose formaldehyde gel.



 $\label{lem:condition} Validation of microRNA.- miR- RTPCR of specific miRNA'S (\ miR-16, miR-21, miR-24, miR26 and miR-103) isolated from mouse tissues. \\ (L=Lung, S= Spleen, H= Heart, B= Brain)$ 

#### GeNei T

#### References:

- BALCELLS I, CIRERA S, BUSK PK: Specific and sensitive quantative RT-PCR of miRNA with DNA primer. BMC Biotechnology 2011, 11:7
- Mráz M, Malinova K, Mayer J, Pospisilova S. MicroRNA isolation and stability in stored RNA samples. Biochem Biophys Res Commun. 2009;390:1–4. doi: 10.1016/j.bbrc.2009.09.061.
- Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci. 2010;101:2087–92.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2124500021730	KT245	MicroRNA Isolation Kit- Cells & tissues, 20 preps

# **Total RNA Isolation Kit- Blood**

#### Description:

Isolation of intact RNA is a prerequisite for the analysis of gene expression. One of the important criteria in the isolation of total RNA is to prevent degradation of the RNA during the isolation procedure. Description: The GeneiPure Total RNA isolation kit- Blood is a fast and easy method for isolation of total cellular RNA from fresh, human and animal whole blood. Multiple blood samples can be processed simultaneously within a short time. The principle is based on lysis of blood cells by two independent procedures, Erythrocyte lysis and leukocyte lysis. This procedure uses selective lysis of erythrocytes to isolate leukocytes, which are the target for RNA isolation. Contaminants and enzyme inhibitors such as haemoglobin and heparin are completely removed, leaving behind purified RNA

#### Unique Features: >

- Spin column technology.
- Quick and simple procedure- recovery of pure and intact RNA within 2 hours.
- No phenol/chloroform extraction or ethanol precipitation.
- Compatible with different blood samples and collection methods.

- Highly pure RNA is obtained with A260/A280 of 2.0-2.1
- Minimal or no genomic DNA contamination.
- RNA obtained is of high quality and can be directly used for all downstream applications

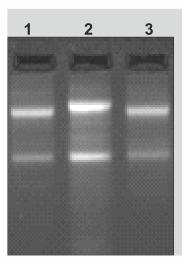
#### Kit Contents:

- Erythrocyte Lysis Buffer (10X)
- Lysis Buffer- Blood
- Wash Buffer I Blood (concentrate)
- Wash Buffer II Blood (concentrate)
- Elution Buffer
- Filtration Columns
- GeneiPure RNA Columns
- Collection Tubes

#### Applications:

#### This RNA ready for use in downstream applications such as:

- RT-PCR and Real time RT-PCR
- cDNA Synthesis
- Northern Blotting
- RNase/S1 Nuclease Protection
- Microarrays

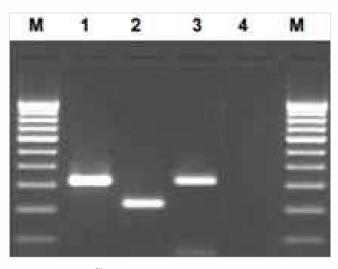


Analysis Total RNA isolated from 1.5 ml of whole blood from different species using GeneiPure Total RNA Isolation Kit-Blood on 1% Agarose Gel.

Lane 1 - Total RNA isolated from Human Blood

Lane 2 - Total RNA isolated from Mouse blood

Lane 3 - Total RNA isolated from Rabbit blood



**Lane M** - StepUp<sup>™</sup>100 bp DNA ladder

Lane 1 - RT-PCR amplification of human GAPDH gene (323 bp)

Lane 2 - RT-PCR amplification of mouse GAPDH (223 bp)

Lane 3 - RT-PCR amplification of rabbit GAPDH gene (315)

Lane 4 - Negative RT-PCR

#### MicroRNA Isolation Kit- Cells & tissues

Analysis of RT-PCR amplified GAPDH gene from 500 ng of total RNA isolated from blood of different species using GeneiPure Total RNA Isolation Kit-Blood on a 2% agarose gel.

#### References:

- Optimized protocol for the extraction of RNA and DNA from frozen whole blood sample stored in a single EDTA tube-Hirotaka Yamagata
- Comparison of protocols and RNA carriers for plasma miRNA isolation.
   Unraveling RNA carrier influence on miRNA isolation-Luis A. Ramón-Núñez
- Debey-Pascher, S. et al. RNA-stabilized whole blood samples but not peripheral blood mononuclear cells can be stored for prolonged time periods prior to transcriptome analysis. J. Mol. Diagn

Biological Source (Yeast (1 ml Culture)	Yield total RNA(µg)
S.cerevisiae	40-50
Pistia pastoris	20-30

#### **Ordering Information**

Cat. No	PI No.	Product Description
2117300021730	KT173	Total RNA Isolation Kit- Blood, 20 preps

#### Total RNA Isolation Kit- Yeast

#### Description:

GeneiPure™ Total RNA Isolation Kit - Yeast provides a rapid and easy method of isolating total RNA from different strains of yeast. Overnight grown yeast cultures are first lysed using an enzyme Lyticase followed by incubating in a lysis buffer containing large volume of chaotropic ions. The purified RNA is ready to be used in downstream applications. The kit provides sufficient reagents for 20 Preps

#### **Unique Features:**

- Recovery of pure and intact RNA in an hour.
- Compatible with wide range of strains.
- Highly pure RNA is obtained with A<sub>260</sub>/A<sub>280</sub> of 2.0-2.1
- ◆ No salt carry over.

#### Kit Contents:

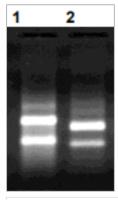
- Lysis Buffer- Yeast
- Wash Buffer I- Yeast (concentrate)
- Wash Buffer II Yeast (concentrate)
- SE Buffer
- Elution Buffer (Nuclease Free water)
- Filtration Columns
- GeneiPure RNA Columns
- Lyticase 2000 U
- Collection Tubes

**Storage:** Lyticase at -20°C Buffers at room temperature (RT).

#### Applications: >

- The purified RNA is ready to be used in downstream applications such as:
- ◆ RT-PCR and Real-time PCR
- cDNA Synthesis
- Northern Blotting
- ◆ RNase/S1 Nuclease Protection
- Micro arrays

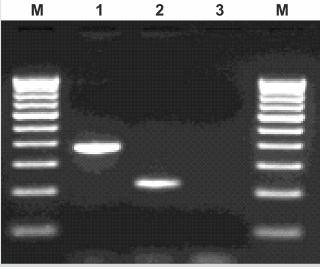
#### **GeNei** TM



Total RNA isolated from different yeast strains using GeneiPure Total RNA Isolation Kit- Yeast. Total RNA was eluted in 50  $\mu$ l and 5  $\mu$ l was loaded on 1% Agarose Gel.

Lane 1 - Saccharomyces cerevisiae

Lane 2 - Pischia pastoris



RT-PCR amplification of 390 bp & 250 bp of ITS3 and ITS4 gene fragment from total RNA isolated from different strains of yeast using GeneiPure Total RNA Isolation KitYeast

Lane M - 100 bp DNA ladder

Lane 1 - Saccharomycescerevisiae

Lane 2 - Pischia pastoris

Lane 3 - Negative control

#### References: >

- Krawetz SA, States JC, Dixon GH. Isolation and fractionation of total nucleic acids from tissues and cells. J Biochem Biophys Methods 1986:12:29–36.
- Wise JA. Preparation and analysis of low molecular weight RNAs and small ribonucleoproteins. Methods Enzymol 1991;194:405–415.
- Koch H, Friesen JD. Individual messenger RNA half-lives in Saccharomyces cerevisiae. Mol Gen Genet 1979;170:129–135.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2117400021730	KT174	Total RNA Isolation Kit- Yeast, 20 preps

#### GeneiPure™ Total RNA Isolation Mini Kit-Plants

#### Description:

Isolation of intact RNA is a prerequisite for the analysis of gene expression. One of the important criteria in the isolation of total RNA is to prevent its degradation during the isolation procedure. The GeneiPure Total RNA Isolation Kit - Plants provides ready to use reagents for isolation of RNA from plant cells and tissues. This kit provides a rapid and simple, silica-based spin column technology to isolate pure and intact RNA within minutes. Plant samples are first lysed and homogenized by incubating in a solution containing large volumes of chaotropic ions. The Lysis Buffer immediately inactivates RNases and creates an appropriate binding condition, which favours the adsorption of RNA to the silica membrane. The washing steps with two different Wash Buffers remove salts, metabolites, and macromolecular cellular components. Pure RNA is eluted under low ionic strength conditions with Elution Buffer.

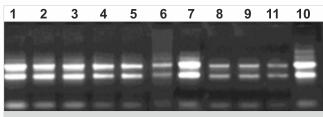
#### Unique Features:

- Spin Column technology.
- Quick and simple procedure- recovery of pure and intact RNA in less than an hour.
- No phenol/chloroform extraction or ethanol precipitation.
- Compatible with wide range of plant tissues and explants.
- Highly pure RNA is obtained with A260/A280 of 2.0-2.1
- Minimal or no genomic DNA contamination.
- RNA obtained is of high quality and can be directly used for all downstream applications.

#### Kit Contents:

- Lysis Buffer- Plants
- Wash Buffer IPlants (concentrate)
- Wash Buffer IIPlants (concentrate)
- Elution Buffer
- Filtration Columns
- GeneiPure RNA Columns
- Collection Tubes

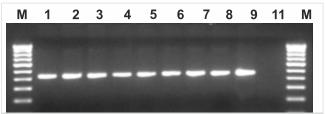
#### Storage: Room Temperature.



Analysis of Total RNA isolated from 50 mg of leaf tissue of different plant species using GeneiPure Total RNA Isolation Kit – Plants on 1% Agarose Gel.

Lane M	-	Tomato	Lane
Lane 1	-	Rice	Lane
Lane 2	-	Maize	Lane
Lane 3	-	Tea	Lane
Lane 4	-	Banana	Lane

Lane 7	-	Papaya
Lane 8	-	Sandal
Lane 9	-	Caconut
Lane 10	-	Arecanut
Lane 11	-	Tobacco



Analysis of RT-PCR amplification of 420 bp Rubisco gene fragment from 500ng of total RNA isolated from leaf tissue of different plant species using GeneiPure Total RNA Isolation Kit – Plants.

#### Applications: >

The purified RNA is ready for use in downstream applications such as:

- ◆ RT-PCR and Real-Time RT-PCR
- ◆ cDNA Synthesis
- Northern Blotting
- RNase/S1 Nuclease Protection
- Microarrays

#### References:

- Rezadoost, M. H., Kordrostami, M. & Kumleh, H. H. An efficient protocol for isolation of inhibitor-free nucleic acids even from recalcitrant plants.
- Wang, L. & Stegemann, J. P. Extraction of high quality RNA from polysaccharide matrices using cetlytrimethylammonium bromide.
- Chang, S., Puryear, J. & Cairney, J. A simple and efficient method for isolating RNA from pine trees.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2117200021730	KT172	GeneiPure™ Total RNA Isolation Mini Kit-Plants, 20 preps
2117200031730	KT172L	GeneiPure ™ Total RNA Isolation Mini Kit- Plants, 50 preps

## GenPro™ 3-in 1 Isolation Kit

## Genpro<sup>™</sup> 3-in-1 Isolation Kit-Cells and Tissues

#### Description: >

Simultaneous extraction of DNA/RNA/Proteins is pre-requisite to study the genome, transcriptome and proteome from a single sample, for gene expression studies, mRNA knockdown, biomarker discovery and characterization of cultured cell lines. One of the important criteria in the isolation of proteins and nucleic acid is to prevent degradation of biomolecules during the isolation procedure. GenPro™ 3-in-1 Isolation Kit - Cells & Tissues provides a rapid and easy method for the simultaneous extraction of Total RNA, genomic DNA and Total protein from the same sample using spin column technology in about 1 hour. This quick, reliable and consistent technique provides a simple and effective way to study protein and nucleic acids simultaneously from cells and tissues without affecting yield and quality. GenPro™ 3-in-1 Isolation Kit - Cells and tissues is suitable for isolating macromolecules from precious samples, such as biopsy materials or small subsets of stem cells without fractionating the samples. The obtained DNA, RNA and protein have been tested for relevant downstream application with minimal cross contamination.

## Unique Features: >

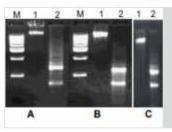
- Spin column technology.
- Quick and simple procedure.
- Sequentially isolate nucleic acids and proteins from single lysate-no need to fractionate the lysate.
- No phenol/chloroform extraction or ethanol precipitation of nucleic acids.
- Compatible with wide range of cells and tissues.
- Suitable for extraction of DNA /RNA and proteins from embryonic stem cell lines.
- High quality total RNA and genomic DNA

# GeNei TM

- High yields of isolated proteins
- DNA/RNA/Protein obtained can be directly used for all downstream applications.

#### Kit Contents:

- Lysis Buffer
- gDNA Wash Buffer I CTB (concentrate)
- RT gDNA Wash Buffer II CTB (concentrate)
- RNA Wash Buffer I CTB (concentrate)
- RNA Wash Buffer II CTB (concentrate)
- DNA Elution Buffer
- RNA Elution Buffer
- ◆ GeneiPure™ DNA Spin Columns
- ◆ GeneiPure™ RNA Spin Columns
- Collection tubes



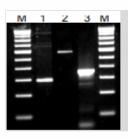
A n a l y s i s of s i m u l t a n e o u s l y extracted DNA and RNA from the same sample using  $GenPro^{TM}$  3-in-1 Isolation Kit-Cells and tissue on Agarose gel.

Lane 1 and 2 represent the DNA and RNA respectively.

- (A) Hela cells
- (B) Mouse Liver tissue
- (C) Mouse embryonic stem cell line Lane M: StepUp $^{\text{TM}}$  1 kb DNA LAdder

## **Ordering Information**

Cat. No	PI No.	Product Description
2117400021730	KT174	Total RNA Isolation Kit- Yeast, 20 preps



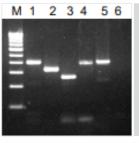
Analysis of PCR amplified genomic DNA isolated from HeLa cells using GenPro TM 3-in-1 Isolation kit-Cells and Tissues.

**Lane M** - StepUp<sup>™</sup> 100 bp DNA Ladder

**Lane 1** - PCR amplification with STR Ioci specific primers

Lane 2 - Alu PCR of plat gene

Lane 3 - PCR amplification with cyt-b primers



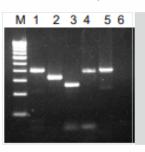
Analysis of RT-PCR amplicons of different genes from Total RNA Isolated from HeLa cells using GenPro $^{\text{TM}}$  3-in-1 Isolation Kit-Cells and Tissues on Agarose gel.

**Lane M** - StepUp<sup>™</sup> 100 bp DNA Ladder

Lane 1 - p53 geneLane 2 - BRCA-2 geneLane 3 - c-myc gene

Lane 4 - Retinoid X receptor G gene (RXRB)

Lane 5 - GAPDH geneLane 6 - Negative RT-PCR



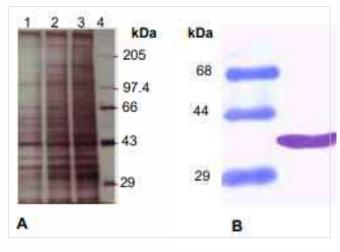
Analysis of RT-PCR amplicons of different genes from Total RNA Isolated from HeLa cells using GenPro™ 3-in-1 Isolation Kit-Cells and Tissues on Agarose gel.

**Lane M** - StepUp<sup>™</sup> 100 bp DNA Ladder

Lane 1 - p53 gene
Lane 2 - BRCA-2 gene
Lane 3 - c-myc gene

Lane 4 - Retinoid X receptor ß gene (RXRB)

Lane 5 - GAPDH geneLane 6 - Negative RT-PCR



- A. Detection of Proteins extracted from HeLa cells using conventional method (Lane 1) and GenPro™ 3-in-1 Isolation Kit-Cells and Tissues (Lane 2 and 3), by silver staining. Lane 4 represents Medium Range Protein Molecular Weight Marker.
- B. Western Blot analysis of the protein extracted from HeLa cells using GenPro™ 3-in-1 Isolation Kit-Cells and Tissues



Sample	DNA (µg)	RNA (µg)	Protein (µg)
Cells (1x10")			
HeLa	6-8	15-18	150-250
NIH-3T3	6-8	8-10	80-100
COS-7	5-8	28-35	80-100
Mouse tissue	es (15 mg)		
Liver	15-20	55-60	350-450
Heart	10-14	6-10	250-300

The average yields of Genomic DNA, Total RNA and proteins obtained from simultaneous extraction of nucleic acids and protein from different cells and tissues samples using  $GenPro^{TM} 3-in-1$  Isolation KitCells and Tissues.

#### Applications:

- gDNA isolated from this kit finds its downstream application PCR, Southern blotting and SNP analysis.
- Total RNA isolated from this kit finds its downstream application, In vitro translation, Northern Blotting, RT-PCR, Real time PCR
- Total Proteins isolated from this kit finds its downstream application, SDS-PAGE, Western Blotting and 2-D Electrophoresis.

#### References:

- Parzer S, Mannhalter C. A rapid method for the isolation of genomic DNA from citrated whole blood. The Biochemical journal 1991;273(Pt 1):229-31 from citrated whole blood. Biochem J 273Pt 11991:229-31
- Sambrook, J. et al., (1989) Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2120400021730	KT204	Genpro™ 3-in-1 Isolation Kit-Cells and Tissues, 20 preps

# Genpro™ 3-in-1 Isolation Kit-Blood

#### Description:

Simultaneous extraction of DNA/RNA/Proteins from the same sample is prerequisite for studying the genome, transcriptome and proteome. One of the important criterion in the isolation of proteins and nucleic acid is to prevent degradation during the isolation procedure. GenPro™ 3-in-1 Isolation Kit provides a rapid and easy method for the simultaneous extraction of total RNA, genomic DNA and Total proteins from the same sample using spin column technology. The Total RNA, genomic DNA and protein are purified in about 1 hour. This quick, reliable and consistent technique provides a simple and effective way to study protein and nucleic acids simultaneously from blood without affecting the yield and quality. GenPro™ 3-in-1 Isolation Kit - Blood is suitable for isolating macromolecules from precious samples without fractionating the samples. The obtained DNA, RNA and protein have been tested for relevant downstream applications without any cross contamination.

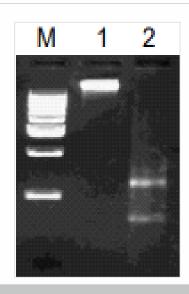
#### Unique Features: >

- Spin column technology.
- Quick and simple procedure.
- Sequentially isolate nucleic acids and proteins from single lysate-no need to fractionate the lysate.
- No phenol/chloroform extraction or ethanol precipitation of nucleic acids.
- Compatible with wide range of cells and tissues.
- Suitable for extraction of DNA /RNA and proteins from embryonic stem cell lines.
- High quality total RNA and genomic DNA
- High yields of isolated proteins
- DNA/RNA/Protein obtained can be directly used for all downstream applications.

#### Kit Contents:

- 10X Erythrocyte Lysis Buffer
- ◆ Lysis Buffer CTB
- Wash Buffer I CTB (concentrate)
- gDNA Wash Buffer II CTB (concentrate)
- Wash Buffer I CTB (concentrate)
- RNA Wash Buffer II CTB (concentrate)
- GeneiPureTMDNA spin Columns
- GeneiPureTMRNA spin Columns
- Collection tubes
- DNA Elution Buffer
- RNA Elution Buffer

# GeNei "

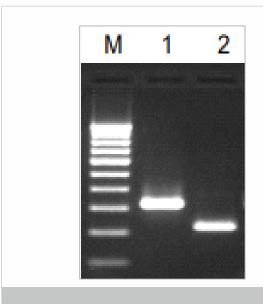


Analysis of DNA and RNA extracted simultaneously from human blood sample using GenProTM 3-in-1 Isolation Kit-Blood on Agarose gel

**Lane M** - StepUp<sup>™</sup>1kb DNA Ladder

Lane 1 - DNA from Human Blood

Lane 2 - RNA from Human Blood



Analysis of DNA and RNA extracted simultaneously from human blood sample using  ${\tt GenProTM\,3-in-1}$  Isolation Kit-Blood on Agarose gel

**Lane M** - StepUp $^{\text{TM}}$  100 bp DNA Ladder

Lane 1 - RT-PCR amplification of human GAPDH gene (323bp)Lane 2 - RT-PCR amplification of mouse GAPDH gene (223bp)

Sample	DNA (ug)	RNA(ug)	Protein (ug)
	Whole	blood (1 m	l)
Human	5-8	4-6	150-180
Mice	3-6	2-3	120-160

Average yields of DNA, RNA and Protein obtained by simultaneous extraction of nucleic acids and protein from blood of different species using GenPro $^{TM}$ 3-in-1Isolation Kit – Blood

#### Applications: >

- gDNA isolated from this kit finds its downstream application PCR, Southern blotting and SNP analysis.
- Total RNA isolated from this kit finds its downstream application, In vitro translation, Northern Blotting, RT-PCR, Real time PCR
- Total Proteins isolated from this kit finds its downstream application, SDS-PAGE, Western Blotting and 2-D Electrophoresis.

#### References:

- Albarino CG. V. Romanowski. Phenol extraction revisited: a rapid method for the isolation and preservation of human genomic DNA from whole blood. Mol Cell Probes 1994;8:423-7.2.
- Parzer S, Mannhalter C. A rapid method for the isolation of genomic DNA from citrated whole blood. The Biochemical journal 1991;273(Pt 1):229-31 from citrated whole blood. Biochem J 273Pt 11991;229-31 4.
- Pochi R, Genomic DNA. Subbarayan, Malancha( et al) Isolation of from Human Whole Blood. BioTechniques December 2002;33 SRC -GoogleScholar:1231-4.

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2120500021730	KT205	Genpro™ 3-in-1 Isolation Kit-Blood, 20 preps

## Genpro™ 3-in-1 Isolation Kit-Plant

## Description: >

Simultaneous extraction of DNA/RNA/Proteins from the same sample is prerequisite for studying the genome, transcriptome and proteome of the sample. One of the important criteria in the isolation of proteins and nucleic acid is to prevent degradation of the biomolecules during the isolation procedure.

GenProTM 3-in-1-Isolation Kit provides a rapid and easy method for the simultaneous extraction of total RNA, genomic DNA and proteins from the same sample, using spin column technology without fractionating the sample in less than 1 hour. This quick, reliable, and consistent technique provides a simple and effective way to study protein and

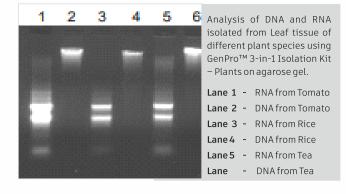
nucleic acids simultaneously from a variety of plant species without affecting yield and quality. The kit is suitable for isolating macromolecules from precious samples without fractionating the samples. The obtained DNA, RNA and protein have been tested for relevant downstream applications without any cross contamination.

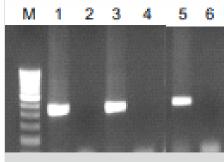
#### Unique Features:

- Parzer S, Mannhalter C. A rapid method for the isolation of genomic DNA from citrated whole blood. The Biochemical journal 1991;273(Pt 1):229-31 from citrated whole blood. Biochem J 273Pt 1 1991:229-31
- Sambrook, J. et al., (1989) Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press

#### Kit Contents:

- Lysis Buffer-BYP
- gDNA Wash Buffer I- BYP (concentrate)
- gDNA Wash Buffer II- BYP (concentrate)
- RNA Wash Buffer I- BYP (concentrate)
- RNA Wash Buffer II- BYP (concentrate)
- DNA Elution Buffer
- RNA Elution Buffer
- GeneiPureTM DNA Spin Columns
- GeneiPureTM RNA Spin Columns 20
- Collection Tubes





Analysis of RT-PCR amplified RUBISCO gene from total RNA of various plant species isolated using GenProTM 3-in-1 Isolation Kit-Plants.

ane	М	-StepUp <sup>™</sup> 100 bp DNA Ladder	Lane 4	- Rice (Negative RT-PCR)
ane	1	-Tomato (Positive RT-PCR)	Lane 5	- Tea (Positive RT-PCR)

Lane 2 -Tomato (Negative RT-PCR) Lane 6 - Tea (Negative RT-PCR)

Lane 3 - Rice (Positive RT-PCR)	RT-PCR)	- Rice (Positive	Lane 3
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Leaf (50 mg)	DNA (ug)	RNA(ug)	Protein (ug)
Human	5-8	4-6	150-180
Mice	3-6	2-3	120-160

Average yields of DNA RNA and proteins obtained from simultaneous extraction of nucleic acids and protein from different plant samples using GenProTM 3-in-1 Kit - Plants.

#### Applications:

- gDNA isolated from this kit finds its downstream application PCR, Southern blotting and SNP analysis.
- Total RNA isolated from this kit finds its downstream application, In vitro translation, Northern Blotting, RT-PCR, Real time PCR
- ◆ Total Proteins isolated from this kit finds its downstream application, SDS-PAGE, Western Blotting and 2-D Electrophoresis.

#### References:

- C. Neal Stewart, Jr., and Laura E. Via. A Rapid CTAB DNA Isolation technique Useful for RAPD Fingerprinting and Other PCR Applications. BioTechniques 1993 article Vol. 14(5):748-749
- Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11-15.
- Doyle, J.J. and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue.
   Focus 12:13-15.
- Mace ES, Buhariwalla HK, and Crouch JH (2003) A high-throughput DNA extraction protocol for tropical molecular breeding programs. Plant Mol Biol Rep 21: 459a-459hMurray M.G. and W.F. Thompson, 1980, Rapid isolation of high molecular weight plant DNA. Nucleic Acid Research, 8 (19), pp. 4321-4325.

Cat. No	PI No.	Product Description
2120600021730	KT206	Genpro™ 3-in-1 Isolation Kit-Plant, 20 preps

# GeNei Th

## Genpro™ 3-in-1 Isolation Kit-Bacteria

#### Description:

Simultaneous extraction of DNA/RNA/Proteins from the same sample is prerequisite for studying the genome, transcriptome, and proteome. One of the important criterion in the isolation of proteins and nucleic acid is to prevent degradation during the isolation procedure.

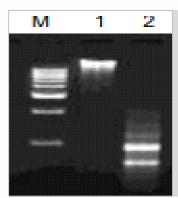
GenPro™ 3-in-1 Isolation Kit provides a rapid and easy method for the simultaneous extraction of total RNA, genomic DNA and proteins from a single sample using spin column technology. Total RNA, genomic DNA and protein are purified in less than 1 hour. This quick, reliable and consistent technique provides a simple and effective way to study protein and nucleic acids simultaneously from different gram-positive and gram-negative bacterial strains without affecting the yield and quality. GenPro™ 3in-1 Isolation Kit - Bacteria is suitable for isolating macromolecules from precious samples without fractionating the samples. The obtained DNA, RNA and protein isolated using this kit, have been tested for relevant downstream applications without any cross contamination.

#### Unique Features: >

- Spin column technology.
- Quick and simple procedure.
- Sequentially isolate nucleic acids and proteins from single lysate-no need to fractionate the lysate.
- No phenol/chloroform extraction or ethanol precipitation of nucleic acids.
- Compatible with wide range of cells and tissues.
- Suitable for extraction of DNA /RNA and proteins from embryonic stem cell lines.
- High quality total RNA and genomic DNA
- High yields of isolated proteins
- DNA/RNA/Protein obtained can be directly used for all downstream applications.

#### Kit Contents:

- Lysozyme
- Lysis Buffer-BYP
- gDNA Wash buffer I- BYP (concentrate)
- gDNA Wash Buffer II-BYP (concentrate)
- RNA Wash Buffer I BYP (concentrate)
- RNA Wash Buffer II BYP (concentrate)
- GeneiPure™ DNA Spin Columns (Green)
- GeneiPure™ RNA Spin Columns (Blue)
- Collection Tubes 40 Nos.
- DNA Elution Buffer
- RNA Elution Buffer



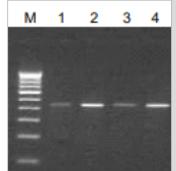
Analysis of DNA and RNA isolated from E.coli culture using GenProTM 3-in-1 Isolation Kit - Bacteria.

Lane M - StepUp™

1 kb DNA LAdder

Lane 1 - DNA from E.coli

Lane 2 - RNA from E.coli



Analysis of RT-PCR amplified 23S rRNA gene from total RNA isolated from different bacterial strains using  $GenPro^{TM}$  3-in-1 isolation Kit-Bacteria.

**Lane M -** StepUp<sup>™</sup> 100 bp DNA ladder

Lane 1 - Bacillus subtilis

Lane 2 - Staphylococcus sp.

Lane 3 - E. coli

Lane 4 - Pseudomonas sp.

Sample	DNA (µg)	RNA (µg)	Protein (µg)	
Bacterial cu	Bacterial cultures (1 ml)			
E.coli	2-3	10-15	200-300	
S.aureus	8-10	50-60	300-600	

Average yields of DNA, RNA and Protein obtained by simultaneous extraction of nucleic acids and protein from different bacterial cultures using GenPro $^{TM}$  3-in-1 Isolation Kit - Bacteria.

#### Applications:

- gDNA isolated from this kit finds its downstream application PCR, Southern blotting and SNP analysis.
- Total RNA isolated from this kit finds its downstream application, In vitro translation, Northern Blotting, RT-PCR, Real time PCR
- Total Proteins isolated from this kit finds its downstream application, SDS-PAGE, Western Blotting and 2-D Electrophoresis.

#### References:

- Preparation of genomic DNA from bacteria K Wilson
- Marmur J. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J Mol Bio. 1961;3(2):208–218. doi: 10.1016/S0022-2836(61)80047-8.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2120800021730	KT208	Genpro™ 3-in-1 Isolation Kit- Bacteria, 20 preps

## **NUCLEASE FREE BUFFERS**

## Tris-Cl 1M

#### Description: >

Tris is the main buffering component; its principal role is to preserve the pH of the buffer at a stable point, usually 8.0. Moreover, tris probable relates with the LPS (lipopolysaccharide) in the membrane, serving to destabilize the membrane further.

Tris-Cl (pH 8.0) is commonly used buffer for molecular biology experiments. This buffer system is markedly temperature dependent. The change in pH per 10° C amounts to approximately 0.3 pH.

Storage: 4°C/Room Temperature

#### Quality control assays: >

**Performance Test:** Routinely used for buffering enzyme preparations, in assay buffers and as rehydrating solution in DNA extraction.

#### Applications: >

- Forms the basic component for the extraction buffer (Edwards buffer),DNA Resuspension (TE),DNA electrophoresis buffer (TAE)
- SDS-PAGE analysis buffer components -Stacking and ruining gel preparations of SDS-PAGE, Towbin buffer (the running buffer for SDS-PAGE)

#### References:

- FISCHER, Beda E.; HARING, Ulrich K.; TRIBOLET, Roger; SIGEL, Helmut (1979). "Metal Ion/Buffer Interactions. Stability of Binary and Ternary Complexes Containing 2-Amino-2(hydroxymethyl)-1,3-propanediol (Tris) and Adenosine 5'-Triphosphate (ATP)"
- https://cshprotocols.cshlp.org/content/2011/2/pdb.rec12394.full

#### **Ordering Information**

Cat. No	PI No.	Product Description
2151581001730	FC50	Tris-Cl 1M (pH 8.0), (DNase, RNase Free) 100 ml

# Saturated Phenol (Water) (Protease, DNase & RNase Free)

#### Description:

Phenol is redistilled and saturated with DEPC water, ready to be equilibrated with required buffer. Contains antioxidant.

- Molecular Weight: 94.11
- Formula: C<sub>6</sub>H<sub>5</sub>OH
- ◆ pH:5.5-6

Application: Used in RNA extractions to remove contaminating proteins.

Storage: 4° C, Protect from light.

Highly corrosive and toxic. Causes severe burns. Handle with care.



#### Quality control assays:

Performance Test: Functional performance is evaluated in nucleic acid extraction and integrity of extracted nucleic acid is determined by gel analysis. Performance tested in RNA extraction.

#### References:

 Smith, Michael B.; March, Jerry (2007), Advanced Organic Chemistry: Reactions, Mechanisms, and Structure

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2151880601730	FC5	Saturated Phenol (Water) (Protease, DNase & RNase Free), 60 ml

## Saturated Phenol (Water)

Phenol is redistilled and saturated with water, ready to be equilibrated with required buffer. Contains antioxidant.

- Molecular Weight: 94.11
- Formula: C<sub>6</sub>H<sub>5</sub>OH
- pH:5.5-6.3

## Applications: >

- Used in DNA and RNA extractions to remove contaminating proteins.
- ◆ Directions to use: For use in DNA extraction, equilibrate with Tris-HCl and adjust pH to 7 – 7.5.

#### Storage: 4°C, Protect from light.

Highly corrosive and toxic. Causes severe burns. Handle with care.

## Quality control assays: >

Performance Test: Functional performance is evaluated in nucleic acid extraction and integrity of extracted nucleic acid is determined by gel analysis.

#### References:

- Smith, Michael B.; March, Jerry (2007), Advanced Organic Chemistry: Reactions. Mechanisms. and Structure
- Phenol extraction of proteins for proteomic studies of recalcitrant plant tissues- Mireille Faurobert 1, Esther Pelpoir, Jamila Chaïb

#### **Ordering Information**

Cat. No	PI No.	Product Description
2151683001730	FC3	Saturated Phenol (Water), 300 ml

## **50X Denhardt's Reagent**

#### Description:

Denhardt's Solution is a mixture of blocking agents used in membrane-based hybridization protocols. The solution contains 1% Ficoll (type 400), 1% polyvinylpyrrolidone, and 1% bovine serum albumin. The solution is filter sterilized and dispensed into sterile bottles.

**Note:** Supplied at 50X concentration. Dilute in Prehybridization or hybridization buffer as per the requirement.

#### Application:

Denhardt's Solution is a blocking reagent for preventing unspecific binding of nucleic acids to nitrocellulose or nylon membranes in hybridisation experiments. Denatured DNA binds to nitrocellulose membranes, pre-treatment of the membrane with Denhardt's solution prevents the binding of single stranded or (unspecific) denatured DNA. However, the specific 'annealing' of denatured DNA to its complementary DNA is notinhibited by this pre-treatment.

Storage: -20°C.

#### Quality control assays:

- Absence of nuclease activity: 1 μg of λ EcoR I digest incubated with 1X Denhardt's Solution, at 37°C for 16 hrs in 50 μl reaction volume, showed sharp unaltered pattern on 1% agarose gel.
- Absence of nickase activity: 1 μg supercoiled plasmid DNA incubated with 1X Denhardt's Solution, at 37°C for 16 hrs in 50 μl reaction volume, showed unaltered pattern without nicking on 1% agarose gel.
- Absence of RNase activity: 1 μg of total RNA incubated for 16 hours at 37°C with 1X Denhardt's Solution, in 20 μl reaction volume, showed no degradation of RNA on 2% agarose gel

#### References:

- https://cshprotocols.cshlp.org/content/2008/12/pdb.rec11538.
- Taub, Floyd (1983). "Laboratory methods: Sequential comparative hybridizations analyzed by computerized image processing can identify and quantitate regulated RNAs

#### **Ordering Information**

Cat. No	PI No.	Product Description
2602980501730	FC78S	50X Denhardt's
		Reagent, 50 ml

## Saturated Phenol (Tris-HCl)

#### Description:

Phenol is redistilled and saturated with Tris-HCl (pH 8.0). Contains antioxidant.

- Molecular Weight: 94.11
- Formula: C<sub>6</sub>H<sub>5</sub>OH
- pH:7.9-8.1

## Applications: >

**Performance Test:** Functional performance is evaluated in nucleic acid extraction and integrity of extracted nucleic acid is determined by gel analysis. Performance tested in RNA extraction.

Storage: 4°C, Protect from light.

Highly corrosive and toxic. Causes severe burns. Handle with care.

#### Quality control assays:

 Performance Test: Functional performance is evaluated in nucleic acid extraction and integrity of extracted nucleic acid is determined by gel analysis

#### References:

- Smith, Michael B.; March, Jerry (2007), Advanced Organic Chemistry: Reactions, Mechanisms, and Structure
- Phenol extraction of proteins for proteomic studies of recalcitrant plant tissues- Mireille Faurobert 1, Esther Pelpoir, Jamila Chaïb

#### **Ordering Information**

Cat. No	PI No.	Product Description
2151780601730	FC3T	Saturated Phenol (Tris-HCl), 60 ml

# Lymphocyte separating solution.

Lymphocyte Separating Solution product is used for in vitro isolation of lymphocytes from peripheral blood. The procedure is based on Boyum's method of rapid separation of lymphocytes using centrifugation through a Ficoll-Sodium diatrizoate solution. Diluted blood is layered over the Ficoll-Sodium diatrizoate solution and centrifuged at a low speed for a short time. Erythrocytes and granulocytes sediment to the bottom of the tube and the mononuclear cells (lymphocytes) and platelets are collected from the interface between the two phases.

Storage: 4°C

#### Quality control assays:

Appearance	Specification	Result
Appearance (Turbidity)	Clear	Clear
Appearance (Form)	Liquid	Liquid
Appearance (Colour)	Colorless	Colorless
Sterility	Pass	Passes
Lymphocyte Separation	Lymphocyte cells to be seen under light microscope after separation.	Passes

#### References:

- A new, fast and convenient method for layering blood or bone marrow over density gradient medium.-A Islam
- Harris R, Ukaejiofo EO. Tissue typing using a routine one-step lymphocyte separation procedure. Br J Haematol. 1970 Feb;18(2):229-235

Cat. No	PI No.	Product Description
2100280501730	FC88M	Lymphocyte separating solution, 50 ml



# O.5M EDTA (DNase, RNase Free)

#### Description:

◆ Ethylenediaminetetraacetic acid (EDTA), is an aminopolycarboxylic acid. This white, watersoluble solid is widely used to bind to iron (Fe2+/Fe3+) and calcium ions (Ca2+), forming water-soluble complexes even at neutral pH. It is thus used to dissolve Fe- and Ca-containing scale as well as to deliver iron ions under conditions where its oxides are insoluble. EDTA is available as several salts, notably disodium EDTA, sodium calcium edetate, and tetrasodium EDTA, but these all function similarly

Ethylenediaminetetraacetic acid, (EDTA) is supplied as an aqueous solution having 0.5 M concentration and pH 8.0.

- Molecular weight: 372.20.
- ◆ pH:8 ± 0.2 at 25°C

#### Application:

• EDTA is a chelator of divalent cations and routinely added in the buffers used for the preparation of nucleic acids, proteins etc.

Storage: 4°C.

#### Quality control assays: >

- Absence of nuclease activity: 1 μg of pUC 18 digest incubated with 10 mM EDTA at 37°C for 16 hrs showed sharp unaltered pattern on 1.2% agarose gel.
- Absence of nickase activity: 1 μg supercoiled plasmid DNA incubated with 10 mM of EDTA at 37°C for 16 hrs showed unaltered pattern without nicking on 1% agarose gel.
- Absence of RNase activity: 1 μg of total RNA incubated for 4 hours at 37°C with 10 mM EDTA in 20 μl reaction volume showed no degradation of RNA on 2% agarose gel.

#### References:

- Norvell, W. A.; Lindsay, W. L. (1969). "Reactions of EDTA Complexes of Fe, Zn, Mn, and Cu with Soils.
- Sinex, Scott A. "EDTA A Molecule with a Complex Story". University of Bristol.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2151280501730	FC43	0.5M EDTA (DNase, RNase Free), 50ml

## 10X TE (DNase, RNase Free)

#### Description:

- ◆ TE buffer is a commonly used buffer solution in molecular biology, especially in procedures involving DNA, cDNA or RNA. "TE" is derived from its components: Tris, a common pH buffer, and EDTA, a molecule that chelates cations like Mg2+. The purpose of TE buffer is to solubilize DNA or RNA, while protecting it from degradation.
- Tris-EDTA, pH 8 is prepared using molecular biology reagent Trizma base and EDTA disodium salt. Product supplied is at 10X concentration (100 mM Tris-HCl and 10 mM EDTA).
- Appearance: Clear colourless solution.
- Store: 4° C/Room Temperature.
- pH Test: 8.0 ± 0.2 at 25°C (1X in water)
- DNase, RNase: None detected

Note: Prepare 1X concentration in double distilled water.

 Used as rehydrating solution in DNA extraction at 1X concentration.

## Applications:

 Yagi N, Satonaka K, Horio M, Shimogaki H, Tokuda Y, Maeda S (1996). "The role of DNase and EDTA on DNA degradation in formaldehyde fixed tissues". Biotechnic & Histochemistry.

Cat. No	PI No.	<b>Product Description</b>
2151381001730	FC44	10X TE (DNase, RNase Free), 100ml

# Sodium Acetate 3M (DNase, RNase Free)

#### Description:

Sodium acetate 3M (pH 5.2) is prepared using molecular biology grade reagents and double distilled water. A solution of sodium acetate (a basic salt of acetic acid) and acetic acid can act as a buffer to keep a relatively constant pH level. This is useful especially in biochemical applications where reactions are pH-dependent in a mildly acidic range (pH 4–6).

• Formula: C2H3O2Na

• Appearance: Clear colourless solution.

pH:5.2 ± 0.2 at 25° C.

#### Applications: >

Used in DNA precipitation.

Storage: Room Temperature.

#### Quality control assays:

- Absence of nuclease activity: 1 μg of pUC 18 digest incubated with 0.3 M Sodium acetate at 37°C for 16 hrs in 50 μl reaction volume, showed sharp unaltered pattern on 1.2% agarose gel.
- Absence of nickase activity: 1 µg supercoiled plasmid DNA incubated with 0.3 M Sodium acetate at 37°C for 16 hrs in 50 µl reaction volume, showed unaltered pattern without nicking on 1% agarose gel.
- Absence of RNase activity: 1 μg of total RNA incubated for 4 hours at 37°C with 0.3 M Sodium acetate in 20 μl reaction volume, showed no degradation of RNA on 2% agarose gel.
- Performance Test: Sodium acetate is routinely used in DNA precipitations.

## References: >

- Acetic acid, sodium salt, hydrate (1:1:3) in Linstrom, Peter J.; Mallard, William G. (eds.); NIST Chemistry WebBook, NIST Standard Reference Database Number 69, National Institute of Standards and Technology, Gaithersburg (MD)
- Hsu, Leh-Yeh; Nordman, C. E. (1983). "Structures of two forms of sodium acetate, Na+.C2H3O2-

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2151481001730	FC45	Sodium Acetate 3M (DNase, RNase Free), 100ml

## **Nuclease Free water**

#### Description:

DEPC-treated water serves as a crucial tool in maintaining the fidelity of RNA-related experiments, where the avoidance of nuclease contamination is paramount for accurate and reliable results. These enzymes pose a potential threat by degrading RNA and DNA, respectively.

DEPC(Diethypyrocarbonate) is used in molecular cloning to inactivate trace amounts of RNases that may contaminate solutions, glassware, and plasticware that are to be used for the preparation of nuclear RNA or mRNA It is a highly reactive alkylating agent that destroys the enzymatic activity of RNase chiefly by ethoxy formylation of histidyl groups. In addition to reacting with histidine residues in proteins, DEPC can form alkalilabile adducts with the imidazole ring N7 of unpaired purines, resulting in cleavage of the glycosidic bond and generation of an alkali-labile a basic site. Because of its high reactivity and specificity, DEPC has been used as a chemical probe of secondary structure in DNA and RNA. Unpaired adenine residues are strongly reactive as are quanine residues in Z-DNA. A diminution in the reactivity of purines with DEPC can therefore be used to measure binding between Z-DNA and specific proteins.

#### Quality control assays:

- Absence of nuclease activity: 1 µg of l DNA digest incubated with 50 µl of water at 37°C for 16 hours showed sharp unaltered pattern on 1% agarose gel.
- Absence of nickase activity: 1 μg supercoiled plasmid DNA incubated with 50 μl of water at 37°C for 16 hours showed unaltered pattern without nicking on 1% agarose gel.

# GeNei TM

 Absence of RNase Activity: 1 μg of total RNA incubated for 4 hours at 37°C with 20 μl water showed no degradation of RNA on 2% agarose gel.

**RNA Isolation:** DEPC-treated water is used in the preparation of reagents for RNA isolation procedures to ensure that the RNA extracted remains intact and free from RNase contamination.

cDNA Synthesis (Reverse Transcription): When synthesizing complementary DNA (cDNA) from RNA templates, **DEPC-treated** water is used to prepare reaction buffers and dilution solutions to prevent RNA degradation during the reverse transcription process.

PCR (Polymerase Chain Reaction): DEPC-treated water is often used in the preparation of PCR reaction mixes to prevent DNA contamination. DNases, if present, can degrade the DNA template, leading to inaccurate or failed PCR amplification.

**Northern Blotting:** In experiments involving Northern blotting, where RNA molecules are transferred to a membrane and probed for specific sequences, DEPC-treated water is used in various steps to maintain RNA integrity.

**RNase-Free Buffer Preparation:** DEPC-treated water is used to prepare various buffers and solutions required for RNA-related experiments to ensure that they are free from RNase contamination.

Storage: Room Temperature

#### References:

- How to Win the Battle with RNase-Michael R. Green and Joseph Sambrook
- https://www.sciencedirect.com/topics/medicine-anddentistry/diethylpyrocarbonate

#### Ordering Information

Cat. No	PI No.	Product Description
2151181001730	FC41L	Water ( DNase, RNase Free), 100ml
2151100011730	FC41J	Water (DNase, RNase Free), 5 x 100 ml

# 3M Potassium acetate, pH 5.5 (DNase,RNase free)

#### Description:

Potassium acetate 3M (pH 5.5) is prepared using molecular biology grade reagents and water. 3M Potassium acetate pH 5.5 (DNase and RNase-free) is a ready-to-use, sterile solution that is free of nucleases.

- ◆ Formula: KC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>
- Appearance: Clear colourless solution.
- pH:5.5 ± 0.2 at 25° C.

Storage: 4°C

#### Applications: >

- Commonly used buffer in the alkaline lysis method of plasmid DNA purification.
- Used for Nucleic acid precipitation
- Precipitation of RNA following in vitro transcription.

## Quality control assays:

- Absence of nuclease activity: 1 μg of Lambda DNA digest incubated with 0.15 M Potassium acetate at 37°C for 16 hrs in 50 μl reaction volume, showed sharp unaltered pattern on 1.2% agarose gel.
- Absence of nickase activity: 1 µg supercoiled plasmid DNA incubated with 0.15 M Potassium acetate at 37°C for 16 hrs in 50 µl reaction volume, showed unaltered pattern without nicking on 1% agarose gel.
- Absence of RNase activity: 1 μg of total RNA incubated for 16 hours at 37°C with 0.15 M Potassium acetate in 20 μl reaction volume, showed no degradation of RNA on 2% agarose gel

#### References:

- Acetic acid, potassium saltin Linstrom, Peter J.; Mallard, William G. (eds.); NIST Chemistry WebBook, NIST Standard Reference Database Number 69, National Institute of Standards and Technology, Gaithersburg (MD) (retrieved 2014-05-18)
- https://chem.nlm.nih.gov/chemidplus/rn/127-08-2

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
3100180501730	FC79M	3M Potassium acetate, pH 5.5 (DNase,RNase free), 50 ml

## AGAROSE GEL ELECTROPHORESIS REAGENTS

## **Agarose**

#### Description:

Agarose, a neutral polysaccharide derived from the cell walls of specific Rhodophyceae algae, often referred to as agarophyte seaweeds, consists of repeating units of D-galactose and 3,6-anhydro-L-galactose. Is not only non-toxic but also boasts specific properties and specifications that render it indispensable as a gelling agent across numerous applications. Its unique chemical structure confers upon agarose the remarkable ability to form robust gels, even at low concentrations. These gels exhibit a macroreticular structure, characterized by an open and adjustable mesh that can be fine-tuned by varying the concentration of agarose.

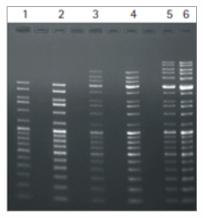
The macro reticule within the agarose gel is primarily shaped by hydrogen bonds, granting it thermo-reversible properties that cause the gel to undergo liquefaction upon heating. What sets agarose apart is its exceptional hysteresis, denoting a significant difference between its gelling and melting temperatures, surpassing other hydrocolloids. Furthermore, the absence of ionic groups imparts a neutral character to the gel, preventing interactions with hydrophilic macromolecules navigating the gel mesh.

## Applications:

• Agarose finds utility in nucleic acid electrophoresis, immunodiffusion techniques and gelplates.

• A prominent application is in gel electrophoresis, a widely used technique in life science laboratories for the separation and detection of biological molecules based on their size, exemplified in DNA separation and analysis.

The quality of Agarose supplied shows the best resolution of the DNA bands on the gel in the below image.



Lane 1 & - DNA Low Range Ruler 0.5 µg and 1 µg
Lane 3 & 4 - DNA Medium Range Ruler 0.5 µg and 1 µg
Lane 5 & 6 - DNA High Range Ruler 0.5 µg and 1 µg

Storage: Room Temperature

#### References:

- https://www.sciencedirect.com/science/article/pii/B9780443069017 500080
- https://pubchem.ncbi.nlm.nih.gov/compound/Agarose'

Cat. No	PI No.	Product Description
2600200101730	FC37	Low Melting Agarose (DNase, RNase Free), 10 g
2600201001730	FC37B	Low Melting Agarose (DNase, RNase Free), 100 g
2600501001730	AGE1	Agarose for Nucleic Acid Electrophoresis- 100g
2600502501730	AGE2	Agarose for Nucleic Acid Electrophoresis- 250g
2600505001730	AGE3	Agarose for Nucleic Acid Electrophoresis- 500g
2600510001730	AGE4	Agarose for Nucleic Acid Electrophoresis- 1000g



# StepUp<sup>™</sup> 50bp ladder

The StepUp™ 50 bp DNA ladder is a quantitative versatile molecular weight marker, featuring a spectrum of 20 bands of double-stranded DNA fragments ranging from 50 to 1000 bp, with a uniform size increment of 50 bp. This ladder is specifically crafted to excel in sizing DNA fragments generated from a number of proprietary plasmids purified by cesium chloride gradient,digested to completion with restriction enzyme, ethanol precipitated

One noteworthy attribute of this DNA ladder is the inclusion of a strategically spiked 500 bp band. This serves as a reference point, enhancing the ease of detection in your experimental analyses. Whether you are engaged in routine DNA fragment sizing or complex molecular biology procedures, the StepUp<sup>TM</sup> 50 bp DNA ladder offers a precise and reliable solution, elevating the accuracy and efficiency of your laboratory work.

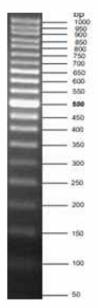
Storage: -20°C.

**Storage Conditions:** Supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA.

**Related Product:** 2663170501730 - RMBD31L StepUp 50bp DNA ladder Ready to use (100 loads)

## Applications:

- Determination of Fragment Sizes: DNA ladders are most used to estimate the size of DNA fragments in an electrophoresis gel.
- PCR Product Verification: After performing polymerase chain reaction (PCR), researchers can use a DNA ladder to confirm that the PCR amplification has produced the expected DNA fragment sizes. This is particularly important when conducting experiments that require the precise sizing of PCR products.
- DNA Purification: When purifying DNA from a gel after electrophoresis, DNA ladders can help identify the target DNA fragments for excision and extraction.
- ◆ The approximate mass of DNA in each of the bands on loading 1µg of StepUp 50bp DNA Ladder.



Fragment	Base Pairs	DNA Mass ng/µg
1	1000	47
2	950	41
3	900	42
4	850	40
5	800	37
6	750	35
7	700	30
8	650	61
9	600	54
10	550	51
11	500	203
12	450	61
13	400	52
14	350	49
15	300	28
16	250	35
17	200	37
18	150	49
19	100	27
20	50	21

 $1~\mu g$  of StepUp 50 bp DNA Ladder visualized by ethidium bromide staining on a 2% agarose gel.

#### References:

- https://microbenotes.com/dna-ladders/
- Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (8 eds.). Cambridge University Press: New York.
- Zhang, G., Fenyö, D., & Neubert, T. A. (2008). Use of DNA ladders for reproducible protein fractionation by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) for quantitative proteomics. Journal of proteome research, 7(2), 678-686. https://doi.org/10.1021/pr700601y

#### Citations: >

Indian Journal of Clinical Biochemistry, 2008 / 23 (2) 123-129 HIGHER
 ALLELES OF APOLIPOPROTEIN B GENE 3' VNTR: RISK FOR GALLSTONE
 DISEASE Manjusha Dixit, Anvesha Srivastava\*, Gourdas Choudhuri\* and
 Balraj Mittal Departments of Genetics and \*Gastroenterology, Sanjay
 Gandhi Postgraduate Institute of Medical Sciences, Lucknow (India)

Cat. No	PI No.	<b>Product Description</b>
2653170501730	MBD31	StepUp™ 50bp DNA Ladder (100 loads), 50 μg
2663170501730	RMBD31L	StepUp™ 50bp DNA Ladder Ready To Use (100 loads), 50 µg

## StepUp<sup>™</sup> 100bp DNA Ladder

The StepUp™ 100 bp DNA ladder is a quantitative molecular marker designed for molecular biology applications. It comprises ten distinct bands of double-stranded DNA fragments, each with varying sizes, ranging from 100 to 1000 base pairs (bp). The increments in size between these bands are precisely 100 bp.

This DNA ladder is particularly useful for determining the sizes of DNA fragments from a number of proprietary purified plasmids, digested to completion with appropriate restriction enzyme, ethanol precipitated and supplied in 10mM Tris.HCL (pH8.0) & 10mM EDTA. When separated on an agarose gel, these ladder bands serve as a reliable reference for estimating the sizes of other DNA fragments in your sample.

Notably, the StepUp™ ladder includes two prominent bands, one at 600 bp and another at 1000 bp. These bands are strategically included as reference points, making it easier to detect and assess the sizes of other DNA fragments in your experiments.

Storage: -20°C

#### References:

2662670501730- RMBD13 - StepUp 100bp DNA Ladder ( 100 Loads)

#### Note:

- StepUp 100 bp DNA Ladder is suitable for use as Quantitative molecular weight standard for agarose gel electrophoresis and not for polyacrylamide gel electrophoresis.
- 100 bp DNA ladder is shipped with 6X gel loading buffer
- Ready to use Ladder is supplied at a concentration of 100 µg/ml premixed with gelloading buffer.
- Recommended loading volume is 5 µl/lane.

#### Applications: >

**Determination of Fragment Sizes:** DNA ladders are most used to estimate the size of DNA fragments in an electrophoresis gel.

PCR Product Verification: After performing polymerase chain reaction (PCR), researchers can use a DNA ladder to confirm that the PCR amplification has produced the expected DNA

fragment sizes. This is particularly important when conducting experiments that require the precise sizing of PCR products.

**DNA Purification:** When purifying DNA from a gel after electrophoresis, DNA ladders can help identify the target DNA fragments for excision and extraction.

Fragment	Base Pairs	DNA Mass	
(18)	1000	209	
2	900	75	700
3	800	93	100
4	700	82	
5	600	150	
6	500	77	500
7	400	74	400
8	300	80	400
9	200	80	500,000
10	100	80	300
visualize	epUp 100 bp d by ethidiu na2%agarose	m bromide	200

#### References:

- https://microbenotes.com/dna-ladders/
- Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (8 eds.). Cambridge University Press: New York.
- Zhang, G., Fenyö, D., & Neubert, T. A. (2008). Use of DNA ladders for reproducible protein fractionation by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) for quantitative proteomics. Journal of proteome research, 7(2), 678-686. https://doi.org/10.1021/pr700601y

#### Citations:

- Detection of l1 cam mutation in a male child with mental retardation M.
   Swarna, M. Sujatha, P. Usha Rani and P.P. Reddy Institute of Genetics and Hospital for Genetic Diseases, Begumpet, Hyderabad-500 016, A.P.,
   India
- Quadruplex PCR for Simultaneous Detection of Serotype, Biotype, Toxigenic Potential, and Central Regulating Factor of Vibrio cholerae Hemant Kumar Khuntia, Bibhuti Bhusan Pal, and Guru Prasada Chhotray\* Regional Medical Research Centre, Chandrasekharpur, Bhubaneswar, Pin-751023, Orissa, India
- Prevalence of periodontal pathogens in coronary atherosclerotic plaque of patients undergoing coronary artery bypass graft surgery-Jaideep Mahendra1 · Little Mahendra2 · Kurian VM3 · Jaishankar K4 · Mythilli R5 1 Associate Professor Dept. of Periodontics, Meenakshi Ammal Dental College, Chennai 2 Lecturer 5 Professor Dept. of Periodontics, Annamalai University, Chidambaram 3 Senior Consultant, Department of Cardiovascular Thoracic Surgery 4 Consultant, Department of Cardiology Madras Medical Mission, Chennai
- Stage-specific Localization and Expression of c-kit in the Adult Human Testis Sreepoorna K. Unni, Deepak N. Modi, Shilpa G. Pathak, Jayesh V. Dhabalia, and Deepa Bhartiya Stem Cell Biology Department (SKU,DB), Molecular Cell Biology Department (DNM), and Neuroendocrinology Department (SGP), National Institute for Research in Reproductive Health, Mumbai, India, and Department of Urology, King Edward Memorial Hospital and Seth Gordhandas Sunderdas Medical College, Mumbai, India (JVD)



PCR-Based Identification and Characterization of Fusarium sp.
 Associated with Mango Malformation M. Arif,1,2 D. R. Pani,2 N. W. Zaidi,1 and U. S. Singh1 1Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar (U. S. Nagar), Uttarakhand 263145, India 2Department of Molecular Biology & Genetic Engineering, G. B. Pant University of Agriculture and Technology, Pantnagar (U. S. Nagar), Uttarakhand 263145, India Correspondence should be addressed to M. Arif, marif181@gmail.com

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2652670501730	MBD13	StepUp™ 100bp DNA Ladder (100 loads), 50 µg
2662671001730	MBD13J	StepUp™ 100 bp DNA Ladder, (200 loads)100 μg
2662670501730	RMBD13	StepUp™ 100 bp DNA Ladder Ready To Use (100 loads), 50 µg

# StepUp<sup>™</sup> 250bp DNA Ladder (100 loads)

The StepUp™ 250 bp DNA ladder is a versatile quantitative molecular weight marker, featuring a spectrum of 20 bands of double-stranded DNA fragments ranging from 250 to 5000 bp, with a uniform size increment of 250 bp. This ladder is specifically crafted to excel in sizing DNA fragments generated through techniques like PCR and restriction digestion, particularly when separated on an agarose gel.

One noteworthy attribute of this DNA ladder is the inclusion of a strategically spiked 2000 bp band. This serves as a reference point, enhancing the ease of detection in your experimental analyses. Whether you are engaged in routine DNA fragment sizing or complex molecular biology procedures, the StepUp $^{\text{TM}}$  250 bp DNA ladder offers a precise and reliable solution, elevating the accuracy and efficiency of your laboratory work.

#### Storage: -20°C

#### Note:

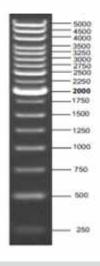
- Stepup 250bp DNA ladder is shipped with 6X gel loading buffer
- StepUp™ 250 bp DNA Ladder is suitable for use as Quantitative molecular weight standard for agarose gel electrophoresis and not for polyacrylamide gel electrophoresis.
- 2000 bp fragment serves as reference band.
- Clear resolved bands are visualized on loading 0.5 1.0 μg of 250 bp DNA Ladder on a 0.8 % agarose gel.

#### References:

2663070501730 - RMBD30L Ready to use Ladder is supplied at a concentration of  $100\,\mu\text{g/ml}$  , premixed with gel loading buffer.

The approximate mass of DNA in each of the bands on loading 1  $\mu g$  of StepUp 250 bp DNA Ladder is as follows

Fragment	Base Pairs	DNA Mass (ng/µg)
1	5000	81
2	4500	73
3	4000	65
4	3500	48
5	3250	53
6	3000	49
7	2750	45
8	2500	. 81
9	2250	36
10	2000	188
11	1750	28
12	1500	45
13	1250	41
14	1000	44
15	750	36
16	500	61
17	250	26



1 µg of StepUp™ 250 bp Ladder visualized by ethidium bromide staining on a 0.8% agarose gel

#### References:

- https://microbenotes.com/dna-ladders/
- Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (8 eds.). Cambridge University Press: New York.
- Zhang, G., Fenyö, D., & Neubert, T. A. (2008). Use of DNA ladders for reproducible protein fractionation by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) for quantitative proteomics. Journal of proteome research, 7(2), 678-686. https://doi.org/10.1021/pr700601y

Cat. No	PI No.	<b>Product Description</b>
2653070501730	MBD30	StepUp™ 250bp DNA Ladder (100 loads), 50µg
2663070501730	RMBD30L	StepUp™ 250bp DNA Ladder Ready To Use (100 loads), 50 µg

bp

# StepUp<sup>™</sup> 500bp DNA Ladder

#### Applications: >

StepUp 500 bp DNA Ladder is a quantitative ladder generated from a number of proprietary plasmids purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzymes, ethanol precipitated and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA.

StepUp 500 bp DNA ladder consists of 10 double stranded DNA fragments of 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 & 5000 base pairs.

- Shipped with 6X gel loading buffer.
- Ready to use Ladder is supplied at a concentration of 100 µg/ml premixed with gel loading buffer.

Recommended loading volume is 5 µl/lane.

#### Features:

- StepUp 500 bp DNA Ladder is suitable for use as quantitative molecular weight standards for agarose gel electrophoresis and not for polyacrylamide gel electrophoresis.
- The 3 kb fragment is ~3 fold more intense to serve as reference band.
- Clear resolved bands are visualized on loading 0.5
   1.0 μg of StepUp 500 bp DNA Ladder on a 0.8% agarose gel.

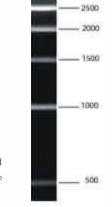
## Quality control assays: >

 Absence of Nuclease Activity: 1 μg of StepUp 500bp DNA Ladder was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 0.8% agarose gel.

Storage: -20C

The approximate mass of DNA in each of the bands on loading 1  $\mu g$  of StepUp 500 bp DNA Ladder is as follows:

Fragment	Base Pairs	DNA Mass
		ng/μg
1	5000	73
2	4500	73
3	4000	73
4	3500	73
5	3000	262
6	2500	76
7	2000	76
8	1500	94
9	1000	94
10	500	106



 $1\,\mu g$  of StepUp 500bp DNA Ladder visualized by ethidium bromide staining on a 0.8% agarose gel.

#### References: >

- https://microbenotes.com/dna-ladders/
- Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (8 eds.). Cambridge University Press: New York.
- Zhang, G., Fenyö, D., & Neubert, T. A. (2008). Use of DNA ladders for reproducible protein fractionation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for quantitative proteomics. Journal of proteome research, 7(2), 678-686. https://doi.org/10.1021/pr700601y

#### Citations:

- Cadmium induces lung inflammation independent of lung cell proliferation: a molecular approach Subhadip Kundu2, Suman Sengupta2, Soumya Chatterjee2, Soham Mitra2 and Arindam Bhattacharyya\*1,2 Address: 1Department of Zoology, University of Calcutta, 35, Ballygange Circular Road, Kolkta-700019, India and 2Department of Environmental Science, University of Kalyani, West Bengal-741235, India
- Regulatory T Cells Suppress T Cell Activation at the Pathologic Site of Human Visceral Leishmaniasis Ambak K. Rai1, Chandreshwar P. Thakur2, Amar Singh1, Tulika Seth3, Sandeep K. Srivastava1, Pushpendra Singh1, Dipendra K. Mitra1\*1 Cellular Immunology Division, Department of T.I.I., All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi, India, 2 Balaji Utthan Sansthan, Patna, Bihar, India, 3 Department of Hematology, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi, India

Cat. No	PI No.	<b>Product Description</b>
2651970501730	MBD19	StepUp™ 500 bp DNA Ladder, 50 μg (100 loads)
2661970501730	RMBD19	StepUp™ 500 bp DNA Ladder, Ready To Use, 50 µg, (100 Loads)



## StepUp<sup>™</sup> 1 kb DNA Ladder

#### Description:

The StepUp 1 kb DNA Ladder is a quantitative ladder generated from several proprietary plasmids purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzyme, ethanol precipitated and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA.

This DNA ladder contains 9 bands of double stranded linear DNA fragments ranging from 1 kb to 10 kb with a size increment of 1 kb. These are suitable for sizing of DNA fragments generated by PCR or restriction digestion, genomic DNA fragments, etc., separated on agarose gel.

#### Storage: -20°C

#### Note:

- Shipped with 6X Gel Loading Buffer.
- Ready to use Ladder is supplied at a concentration of 100 μg/ml premixed with gel loading buffer.
- Recommended loading volume is 5 μl/lane.

#### Features:

- StepUp 1 kb DNA Ladder is suitable for use as quantitative molecular weight standard for agarose gel electrophoresis and not for polyacrylamide gel electrophoresis.
- ◆ The 3 kb fragment is ~2.5 fold more intense to serve as reference band.
- Clear resolved bands are visualized on loading 0.5
   1.0 μg of StepUp 1 kb DNA ladder on a 0.8 1% agarose gel.
- Approximate concentration of DNA can be determined based on visual estimation.

## Quality control assays:

Absence of Nuclease Activity: 1  $\mu$ g of StepUp 1 kb DNA Ladder was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 0.8% agarose gel.

Store: -20C

The approximate mass of DNA in each of the bands on loading 1  $\mu$ g of StepUp 500 bp DNA Ladder is as follows:

Fragment	Base Pairs	DNA Mass ng/pg
1	10000	91
2	8000	91
3	7000	91
4	6000	91
5	5000	91
6	4000	91
7	3000	228
8	2000	113
9	1000	113

 $1\,\mu g$  of StepUp 1 kb DNA Ladder visualized by ethidium bromide staining on a 0.8% agarose gel.

#### References:

- https://microbenotes.com/dna-ladders/
- Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (8 eds.). Cambridge University Press: New York.
- Zhang, G., Fenyö, D., & Neubert, T. A. (2008). Use of DNA ladders for reproducible protein fractionation by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) for quantitative proteomics. Journal of proteome research, 7(2), 678-686. https://doi.org/10.1021/pr700601y

#### Citations:

- Cadmium induces lung inflammation independent of lung cell proliferation: a molecular approach Subhadip Kundu2, Suman Sengupta2, Soumya Chatterjee2, Soham Mitra2 and Arindam Bhattacharyya\*1,2 Address: 1Department of Zoology, University of Calcutta, 35, Ballygange Circular Road, Kolkta-700019, India and 2Department of Environmental Science, University of Kalyani, West Bengal-741235, India
- Regulatory T Cells Suppress T Cell Activation at the Pathologic Site of Human Visceral Leishmaniasis Ambak K. Rai1, Chandreshwar P. Thakur2, Amar Singh1, Tulika Seth3, Sandeep K. Srivastava1, Pushpendra Singh1, Dipendra K. Mitra1\*1 Cellular Immunology Division, Department of T.I.I., All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi, India, 2 Balaji Utthan Sansthan, Patna, Bihar, India, 3 Department of Hematology, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi, India

Cat. No	PI No.	Product Description
2652070501730	MBD20	StepUp™ 1 kb DNA Ladder, 50 μg, (100 loads)
2662070501730	RMBD20	StepUp™ 1 kb DNA Ladder Ready To Use, 50 µg, (100 loads)



## **Supermix DNA Ladder**

#### Description:

Supermix DNA ladder is generated from a number of proprietary plasmids and lambda DNA purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzymes, phenol extracted and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA.

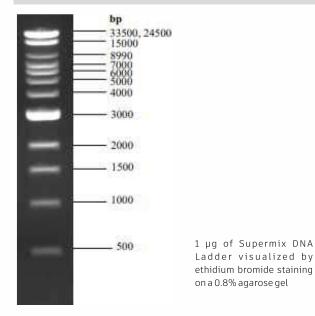
Supermix DNA ladder consists of 13 double stranded DNA fragments of 500, 1000, 1500, 2000, 3000, 4000, 5000, 6000, 7000, 8990, 15000, 24500 & 33500 base pairs.

Shipped with 6X gel loading buffer.

#### Storage: -20°C.

#### Note:

- Supermix DNA Ladder is suitable for use as molecular weight standards for agarose gel electrophoresis.
- Not intended for use in quantitative analysis.
- The 3 kb fragment is 2.5 fold more intense to serve as reference band.
- Clear resolved bands are visualized on loading 0.5 1.0 μg of Supermix DNA ladder on a 0.8% agarose gel.



## Quality control assays: >

Absence of Nuclease Activity: 1  $\mu g$  of Supermix DNA Ladder was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 0.8% agarose gel.

#### References:

- https://microbenotes.com/dna-ladders/
- Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (8 eds.). Cambridge University Press: New York.
- Zhang, G., Fenyö, D., & Neubert, T. A. (2008). Use of DNA ladders for reproducible protein fractionation by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) for quantitative proteomics. Journal of proteome research, 7(2), 678-686. https://doi.org/10.1021/pr700601y

#### **Ordering Information**

Cat. No	PI No.	Product Description
2652171001730	MBD21J	Supermix DNA Ladder, 100 μg, (200 loads)

## 20 bp DNA Ruler

#### Description:

20 bp DNA ruler is generated from several proprietary plasmids purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzyme, phenol extracted and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. 20 bp DNA ruler contains 20 individual double stranded, blunt-ended, linear DNA fragments containing ~ 50% GC – rich sequences of 100, 120, 140, 160, 180, 200, 240, 280, 320, 360, 400, 500, 600, 700, 800, 900, 1000, 1100, 1300 & 1500 bp. Shipped with 6X gel loading buffer.

#### Note:

- 20 bp DNA ruler is suitable for use as Quantitative molecular weight standard for agarose gel electrophoresis and for polyacrylamide gel electrophoresis.
- The ruler can be run on Agarose gel (2-3%) or native polyacrylamide gel (8-10%)
- The lower bands in agarose gel might appear slightly diffused while polyacrylamide gel are suitable for better resolution of the fragments
- To avoid frequent freeze-thaw cycles, store as aliquots at -20° C.

Storage: -20C

Absence of Nuclease Activity: 1  $\mu$ g of 20 bp DNA Ruler was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 2% agarose gel

# GeNei <sup>T</sup>

Fragment	Base Pairs	DNA Mass ng/µg	=
1	1500	112	
2	1300	104	
3	1100	91	
4	1000	81	Section 2
5	900	67	
6	800	64	-
7	700	58	
8	600	48	_
9	500	37	
10	400	32	
11	360	29	-
12	320	26	-
13	280	23	
14	240	19	
15	200	33	-
16	180	28	
17	160	25	
18	140	23	10000
19	120	20	-
20	100	80	

 $1\,\mu g$  of 20 bp DNA Ruler visualized by ethidium bromide staining on a 2% agarose gel

#### References:

- Roberts, M. A.; Crawford, D. L. (1 June 2000). "Use of Randomly Amplified Polymorphic DNA as a Means of Developing Genus- and Strain-Specific Streptomyces DNA Probes"
- Higgins, L. (April 2012). "DNA Ladder for Gel Electrophoresis". Lewis & Clark College

#### Related Product:

2662470501730 - RMBD24 - 20 bp DNA Ruler, Ready to use, (100 Loads)

## **Ordering Information**

Cat. No	PI No.	Product Description
2652470501730	MBD24	20 bp DNA Ruler, 50 μg, 100 loads
2662470501730	RMBD24	20 bp DNA Ruler Ready To Use, 50 μg,100 loads

# Low Range DNA Ruler (100 bp - 3 kb)

## Description:

Low Range DNA Ruler is a quantitative ruler generated from a number of proprietary plasmids purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzymes, ethanol precipitated and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. Low Range DNA Ruler

consists of 9 double stranded DNA fragments of 100, 200, 300, 600, 1000, 1500, 2000, 2500 & 3000 base pairs. Shipped with 6X gel loading buffer.

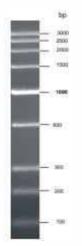
#### Note:

- Low Range DNA Ruler is suitable for use as quantitative molecular weight standards for agarose gel electrophoresis
- 1kb fragment is ~2 fold more intense, to serve as reference band.
- Clear resolved bands are visualized on loading 0.5 1.0 μg of Low Range DNA Ruler on a 1.5 % agarose gel.
- To avoid frequent freeze-thaw cycles, store as aliquots at -20°C.

Storage: -20C

#### Quality control assays: >

 Absence of Nuclease Activity: 1 μg of Low Range DNA Ruler was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 1.5% agarose gel.



Fragment	Base Pairs	DNA Mass ng/µg
14°	3000	78
2	2500	82
3	2000	84
4	1500	88
5	1000	196
6	600	118
7	300	118
8	200	118
9	100	118

 $1\,\mu g$  of Low Range DNA Ruler visualized by ethidium bromide staining on a 1.5% agarose

#### References:

- A. Cooney, "Techniques and high resolution DNA size markers for pulsed field gel electrophoresis," Molecular Biotechnology, vol. 2, no. 2, pp. 119–127, 1994.
- R. C. Parker, R. M. Watson, and J. Vinograd, "Mapping of closed circular DNAs by cleavage with restriction endonucleases and calibration by agarose gel electrophoresis," Proceedings of the National Academy of Sciences of the United States of America, vol. 74, no. 3, pp. 851–855, 1977.
- S. V. Polyarush, S. S. Egamberdiev, D. R. Mansurov, and S. S. Azimova, "Preparation of DNA markers based on E. coli plasmid DNA," Chemistry of Natural Compounds, vol. 39, no. 6, pp. 592–594, 2003.

Cat. No	PI No.	<b>Product Description</b>
2652370501730	MBD23	Low Range DNA Ruler (100 bp - 3 kb), 50 μg, (100 loads)

# Low Range DNA Ruler plus (100 bp - 3kb)

#### Description:

Low Range DNA Ruler Plus is a quantitative ruler generated from a number of proprietary plasmids purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzymes, ethanol precipitated and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. Low Range DNA Ruler Plus consists of 16 double stranded DNA fragments of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1185, 1500, 1815, 2000, 2500 & 3000 base pairs. Shipped with 6X gel loading buffer.

#### Note:

- Low range DNA Ruler Plus is suitable for use as quantitative molecular weight standards for agarose gel electrophoresis
- The 600 and 1000 fragments are 2.5 fold more intense to serve as reference bands.
- Clear resolved bands are visualized on loading 0.5–1.0  $\mu g$  of Low range DNA Ruler Plus on 1% agarose gel.
- To avoid frequent freeze-thaw cycles, store as aliquots at -20° C

Approximate mass of DNA in each of the bands on loading  $1 \mu g$  of low Range DNA Ruler is as follows

3000 2500	Fragment	Base Pairs	DNA Mass ng/µg
	1	3000	49
i	2	2500	49
	3	2000	50
	4	1815	80
ñ	5	1500	53
	6	1185	52
ij	7	1000	139
ij	8	900	50
	9	800	62
Ī	10	700	56
- 8	- 11	600	100
	12	500	51
- 1	13	400	50
	14	300	53
Ü	15	200	53
- 3	16	100	53

 $1~\mu g$  of Low Range DNA Ruler Plus visualized by ethidium bromide staining on a 1% agarose gel

## Quality control assays:

 Absence of Nuclease Activity: 1 μg of Low Range DNA Ruler Plus was incubated with Genei Buffer for 16 hours at 37° C. Sharp unaltered band pattern was seen without smear on a 1% agarose gel

#### References: >

- A. Cooney, "Techniques and high resolution DNA size markers for pulsed field gel electrophoresis," Molecular Biotechnology, vol. 2, no. 2, pp. 119–127. 1994.
- R. C. Parker, R. M. Watson, and J. Vinograd, "Mapping of closed circular DNAs by cleavage with restriction endonucleases and calibration by agarose gel electrophoresis," Proceedings of the National Academy of Sciences of the United States of America, vol. 74, no. 3, pp. 851–855, 1977.
- S. V. Polyarush, S. S. Egamberdiev, D. R. Mansurov, and S. S. Azimova, "Preparation of DNA markers based on E. coli plasmid DNA," Chemistry of Natural Compounds, vol. 39, no. 6, pp. 592–594, 2003.

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2652770501730	MBD27	Low Range DNA Ruler Plus (100 bp - 3 kb), 50 µg, (100 loads)
2662770501730	RMBD27	Low Range DNA Ruler Plus (100 bp - 3 kb) Ready To Use, 50 µg, (100 loads)

## Medium Range DNA Ruler (100 bp - 5 kb)

#### Description:

Medium Range DNA Ruler is a quantitative ruler generated from several proprietary plasmids purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzymes, ethanol precipitated and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. Medium Range DNA Ruler consists of 19 double stranded DNA fragments of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1185, 1500, 1815, 2000, 2500, 3000, 3500, 4000 & 5000 base pairs. Shipped with 6X gel loading buffer.

#### Related Product: RMBD28:

Ready to use Ruler is supplied at a concentration of 100  $\mu$ g/ml premixed with gel loading buffer. Recommended loading volume is 5  $\mu$ l/lane.

#### Storage: -20C

#### Note:

- Medium Range DNA Ruler is suitable for use as quantitative molecular weight standards for agarose gel electrophoresis
- The 600,1000 and 3000bp fragments are ~2.3 fold more intense to serve as reference bands.
- Clear resolved bands are visualized on loading 0.5–1.0 μg of Medium Range DNA Ruler on 1% agarose gel.
- To avoid frequent freeze-thaw cycles, store as aliquots at -20°C.



#### Quality control assays: >

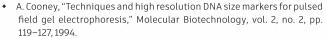
·Absence of Nuclease Activity:  $1 \mu g$  of Medium Range DNA Ruler was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 1% agarose gel.

Approximate mass of DNA in each of the bands on loading 1  $\mu g$  of Medium Range DNA Ruler is as follows

5800	Fragment	Base Pairs	DNA Mass
500	. 81	5000	30
, [	2	4000	30
0	3	3500	33
00	4	3000	101
15	5	2500	34
100	. 6	2000	34
Ī	7	1815	53
5	8	1500	35
10	9	1185	35
0	10	1000	129
	11	900	46
	12	800	52
0	13	700	51
0	14	600	92
100	15	500	47
	16	400	46
00	17	300	49
	18	200	49
100	19	100	49

1  $\mu g$  of Medium Range DNA Ruler visualized by ethidium bromide staining on a 1% agarose gel

## References:



- R. C. Parker, R. M. Watson, and J. Vinograd, "Mapping of closed circular DNAs by cleavage with restriction endonucleases and calibration by agarose gel electrophoresis," Proceedings of the National Academy of Sciences of the United States of America, vol. 74, no. 3, pp. 851–855, 1977.
- S. V. Polyarush, S. S. Egamberdiev, D. R. Mansurov, and S. S. Azimova, "Preparation of DNA markers based on E. coli plasmid DNA," Chemistry of Natural Compounds, vol. 39, no. 6, pp. 592–594, 2003.

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2652870501730	MBD28	Medium Range DNA Ruler (100 bp - 5 kb), 50 μg, (100 loads)
2662870501730	RMBD28	Medium Range DNA Ruler (100 bp - 5 kb) Ready To Use, 50 μg, (100 loads)

# High Range DNA Ruler (100 bp - 10 kb)

#### Description:

High Range DNA Ruler is a quantitative ruler generated from a number of proprietary plasmids purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzymes, ethanol precipitated and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. High Range DNA Ruler consists of 21 double stranded DNA fragments of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1185, 1500, 1815, 2000, 2500, 3000, 3500, 4000, 5000, 6000, 8000 &10000 base pairs. Shipped with 6X gel loading buffer.

#### Note:

- High Range DNA Ruler is suitable for use as quantitative molecular weight standards for agarose gel electrophoresis
- The 600, 1000 and 3000bp fragments are ~2-3 fold more intense to serve as reference bands.
- Clear resolved bands are visualized on loading 0.5–1.0 μg of High Range DNA Ruler on 1% agarose gel.
- To avoid frequent freeze-thaw cycles, store as aliquots at -20° C.

#### Storage: -20C

Approximate mass of DNA in each of the bands on loading 1  $\mu g$  of High Range DNA Ruler is as follows

	bp
	10000 8000 6000 5000
	4000
	3000
_	2500
_	2000
_	1815
	1500
	1185
	1000 900 800
	700
	600
_	500
_	400
	300
_	200
_	100

Fragment	Base Pairs	DNA Mass
1	10000	30
2	8000	30
3	6000	30
4	5000	30
5	4000	30
6	3000	106
7	2500	29
8	2000	31
9	1815	37
10	1500	33
11	1185	24
12	1000	123
13	900	44
14	800	55
15	700	48
16	600	89
17	500	46
18	400	44
19	300	47
20	200	47
21	100	47

 $1~\mu g$  of High Range DNA Ruler visualized by ethidium bromide staining on a 1% agarose gel

#### References:

- A. Cooney, "Techniques and high resolution DNA size markers for pulsed field gel electrophoresis," Molecular Biotechnology, vol. 2, no. 2, pp. 119–127, 1994.
- R. C. Parker, R. M. Watson, and J. Vinograd, "Mapping of closed circular DNAs by cleavage with restriction endonucleases and calibration by agarose gel electrophoresis," Proceedings of the National Academy of Sciences of the United States of America, vol. 74, no. 3, pp. 851–855, 1977.
- · S. V. Polyarush, S. S. Egamberdiev, D. R. Mansurov, and S. S. Azimova, "Preparation of DNA markers based on E. coli plasmid DNA," Chemistry of Natural Compounds, vol. 39, no. 6, pp. 592–594, 2003.
- A. Cooney, "Techniques and high resolution DNA size markers for pulsed field gel electrophoresis," Molecular Biotechnology, vol. 2, no. 2, pp. 119–127, 1994.
- R. C. Parker, R. M. Watson, and J. Vinograd, "Mapping of closed circular DNAs by cleavage with restriction endonucleases and calibration by agarose gel electrophoresis," Proceedings of the National Academy of Sciences of the United States of America, vol. 74, no. 3, pp. 851–855, 1977.
- S. V. Polyarush, S. S. Egamberdiev, D. R. Mansurov, and S. S. Azimova, "Preparation of DNA markers based on E. coli plasmid DNA," Chemistry of Natural Compounds, vol. 39, no. 6, pp. 592–594, 2003.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2652970501730	MBD29	High Range DNA Ruler (100 bp - 10 kb), 50 μg, (100 loads)

# Quantum™ PCR Marker Low Range Ready to Use

## Description:

The Quantum™ PCR Marker Low Range is a quantitative Marker generated from a proprietary plasmid purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzyme, ethanol precipitated and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA.

The Quantum<sup>™</sup> PCR Marker (Low Range) consists of 6 double stranded DNA fragments ranging from (50-1900 bp). The 1900, 900, 550, 300 and 100 bp are prominently visible and useful in quantification. Ready to use Marker is supplied at a concentration of 100 µg/ml premixed with gel loading buffer.

#### Note:

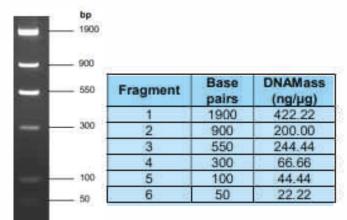
- Quantum™ PCR Marker Low Range is suitable for use as Quantitative molecular weight standard for agarose gel electrophoresis and not for polyacrylamide gel electrophoresis.
- Clear resolved bands are visualized on loading 0.5 − 1.0 µg of Quantum™ PCR Marker (Low Range) on a 1.5 % agarose gel.
- The 6th band of 50bp is faint and may not be visible.
- To avoid frequent freeze-thaw cycles, store as aliquots at -20° C.

Storage: -20C

#### Quality control assays:

Absence of Nuclease Activity: 1  $\mu$ g of Ready to use Quantum<sup>TM</sup> PCR Marker Low Range was incubated for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 1.5% agarose gel.

Approximate mass of DNA in each of the bands on loading 1  $\mu$ g of High Range DNA Ruler is as follows



 $1\,\mu g$  of Marker visualized by ethidium bromide staining on a 1.5 % agarose qel

#### References:

- A. Cooney, "Techniques and high resolution DNA size markers for pulsed field gel electrophoresis," Molecular Biotechnology, vol. 2, no. 2, pp. 119–127, 1994.
- R. C. Parker, R. M. Watson, and J. Vinograd, "Mapping of closed circular DNAs by cleavage with restriction endonucleases and calibration by agarose gel electrophoresis," Proceedings of the National Academy of Sciences of the United States of America, vol. 74, no. 3, pp. 851–855, 1977.
- S. V. Polyarush, S. S. Egamberdiev, D. R. Mansurov, and S. S. Azimova, "Preparation of DNA markers based on E. coli plasmid DNA," Chemistry of Natural Compounds, vol. 39, no. 6, pp. 592–594, 2003.

Cat. No	PI No.	<b>Product Description</b>
2663270501730	RMBD32L	Quantum™ PCR Marker Low Range Ready To Use (100 loads), 50 μg



# Quantum™ PCR Marker Medium Range Ready to Use

#### Description:

The Quantum™ PCR Marker Medium Range is a quantitative marker generated from a proprietary plasmid purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzyme, ethanol precipitated and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA.

The Quantum™ PCR Marker (Medium Range) consists of 5 double stranded DNA fragments ranging from (100 - 4500 bp). The 4500, 2000, 1000 and 400 bp are prominently visible and useful in quantification. Shipped with 6X gel loading buffer.

#### Note:

- Quantum<sup>™</sup> PCR Marker Medium Range is suitable for use as Quantitative molecular weight standard for agarose gel electrophoresis and not for polyacrylamide gel electrophoresis.
- Clear resolved bands are visualized on loading 0.5 - 1.0 μg of Quantum™ PCR Marker (Medium Range) on a 1 % agarose gel.
- The 5th band of 100bp is faint and may not be visible on loading 0.5-1µq of Quantum™ PCR Marker (Medium Range).
- To avoid frequent freeze-thaw cycles, store as aliquots at -20°C.

#### Related Product: RMBD28:

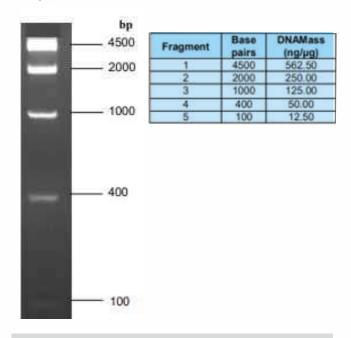
The Quantum<sup>™</sup> PCR Marker (Medium Range) is a quantitative marker generated from a proprietary plasmid purified by Cesium Chloride gradient, digested to completion with appropriate restriction enzyme, ethanol precipitated and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA.

The Quantum™ PCR Marker (Medium Range) consists of 5 double stranded DNA fragments ranging from (100 - 4500 bp). The 4500, 2000, 1000 and 400 bp are prominently visible and useful in quantification. Shipped with 6X gel loading buffer.

#### Quality control assays:

Absence of Nuclease Activity: 1 µg of Quantum™ PCR Marker Medium Range was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 1% agarose gel.

Approximate mass of DNA in each of the bands on loading 1  $\mu$ g of Quantum PCR Marker (Medium Range) is as follows



 $1\,\mu g$  of Marker visualized by ethidium bromide staining on a  $1\,\%$  agarose gel

#### References:

- A. Cooney, "Techniques and high resolution DNA size markers for pulsed field gel electrophoresis," Molecular Biotechnology, vol. 2, no. 2, pp. 119–127, 1994.
- R. C. Parker, R. M. Watson, and J. Vinograd, "Mapping of closed circular DNAs by cleavage with restriction endonucleases and calibration by agarose gel electrophoresis," Proceedings of the National Academy of Sciences of the United States of America, vol. 74, no. 3, pp. 851–855, 1977.
- S. V. Polyarush, S. S. Egamberdiev, D. R. Mansurov, and S. S. Azimova, "Preparation of DNA markers based on E. coli plasmid DNA," Chemistry of Natural Compounds, vol. 39, no. 6, pp. 592–594, 2003.

Cat. No	PI No.	Product Description
2653370501730	MBD33	Quantum™ PCR Marker Medium Range (100 loads), 50µg

# Lambda DNA/EcoR I Digest, Ready to Use

#### Description:

- DNA is completely digested with EcoR I yielding 6 fragments of 21226, 7421, 5804, 5643, 4878 and 3530 base pairs. Heating the digest at 65°C for 5 mins and rapid cooling on ice will ensure the presence of the 3530 base pair fragment.
- Ready to use Marker is supplied at a concentration of 100 μg/ml premixed with gel loading buffer.

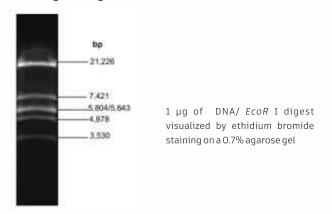
#### Storage: -20C

#### Note:

- The marker is supplied as single aliquot.
- To avoid frequent freeze-thaw cycles, store as aliquots at -20°C.

#### **Quality Assays:**

Absence of Nuclease Activity: 1  $\mu$ g of DNA / EcoR I digest was incubated for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 0.7% agarose gel.



#### References:

 Bacteriophage lambda DNA: the beginning of the end.-A Becker and H Murialdo.

## **Ordering Information**

Cat. No	PI No.	Product Description
2660170501730	RMBD1	Lambda DNA/ <i>EcoR</i> I Digest, Ready To Use, 50 µg, 100 loads

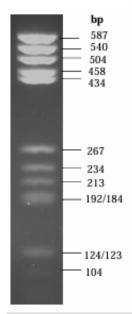
# pBR322 DNA / Hae III Digest

#### Description:

• pBR322 plasmid DNA was purified by cesium chloride gradient, digested to completion with *Hae* III, phenol extracted and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. pBR322 DNA / *Hae* III digest consists of 22 double stranded DNA fragments of 587, 540, 504, 458, 434, 267, 234, 213, 192, 184, 124, 123, 104, 89, 80, 64, 57, 51, 21, 18, 11 and 7 base pairs.

#### **Quality Assays:**

Absence of Nuclease Activity: 1  $\mu g$  of pBR322 / Hae III digest was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 2% agarose gel.



 $1~\mu g$  of pBR322 / Hae III digest visualized by ethidium bromide staining on a 2% agarose gel

Storage: -20C

Cat. No	PI No.	Product Description
2650870501730	MBD8L	pBR322 DNA / Hae III Digest, 50µg, (100 Loads)



# pBR322 DNA/Msp I Digest

#### Description:

pBR322 plasmid DNA was purified by cesium chloride gradient, digested to completion with Msp I, phenol extracted and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. pBR322 DNA / Msp I digest consists of 26 double stranded DNA fragments of 622, 527, 404, 309, 242, 238, 217, 201, 190, 180, 160, 160, 147, 147, 123, 110, 90, 76, 67, 34, 34, 26, 26, 15, 9 & 9 base pairs.

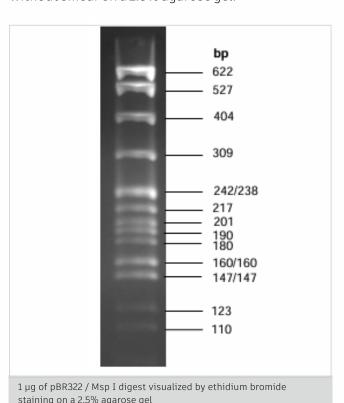
#### Note:

- pBR322 / Msp I digest is suitable for use as molecular weight standards for agarose gel electrophoresis.
- Clear resolved bands are visualized on loading 1.0 µg of pBR322 / Msp I digest on a 2.5% agarose gel.
- Shipped with 6X gel loading buffer.
- To avoid frequent freeze-thaw cycles, store as aliquots at -20°C.

#### Storage: -20C

#### Quality control assays:

Absence of Nuclease Activity:  $1 \mu g$  of pBR322 / Msp I digest was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 2.5% agarose gel.



#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2651070501730	MBD10L	pBR322 DNA/ <i>Msp</i> I Digest,50 μg, (100 loads)

## X174 / Hae III Digest



#### Description:

 $\phi$ X174 plasmid DNA was purified by cesium chloride gradient, digested to completion with Hae III, ethanol precipitated and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. X174 / Hae III digest consists of 11 double stranded DNA fragments of 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118 & 72 base pairs.

#### Note:

- $\varphi$  X174 / Hae III digest is suitable for use as molecular weight standards for agarose gel electrophoresis.
- φXClear resolved bands are visualized on loading 1.0 μg of X174 /
   Hae III digest on a 2% agarose gel.
- Shipped with 6X gel loading buffer
- To avoid frequent freeze-thaw cycles, store as aliquots at -20° C.

#### Related product:-RMBD5:>

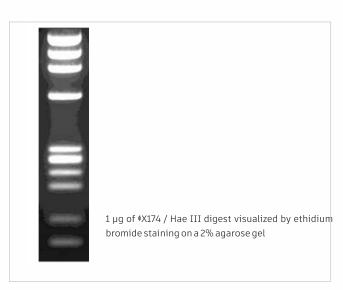
Ready to use Marker is supplied at a concentration of 100  $\mu$ g/ml premixed with gel loading buffer.

As the fragments are present in molar ratio, the smaller fragments will be visible only when >1  $\mu g$  of marker is loaded on gel.

Storage: -20C

#### Quality control assays:

Absence of Nuclease Activity: 1  $\mu$ g of X174 / Hae III digest was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 2% agarose gel.



#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2660570101730	RMBD5	PhiX174 DNA/Hae III Digest Ready To Use, 50 µg, (100 loads)
2650570501730	MBD5	PhiX174 DNA/Hae III Digest, 50 μg, (100 loads)

# Lambda DNA/EcoR I Digest

## Description:

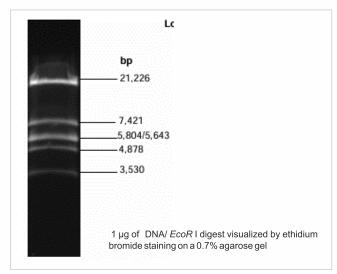
◆ DNA was purified by cesium chloride gradient, digested to completion with *EcoR* I, phenol extracted and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. DNA / *EcoR* I digest consists of 6 double stranded DNA fragments of 21226, 7421, 5804,5643,4878 and 3530 base pairs.

#### Note: DNA / EcoR I

- This DNA digest is suitable for use as molecular weight standards for agarose gel electrophoresis.
- Heating the digest at 65oC for 5 mins and rapid cooling on ice will ensure the presence of the 3530 base pair fragment.
- Clear resolved bands are visualized on loading 1.0
   µg of DNA/EcoRI digest on a 0.7 % agarose gel.
- Cannot be used for ligation experiments.
- · Shipped with 6X gel loading buffer
- To avoid frequent freeze-thaw cycles, store as aliquots at -20°C.

#### Quality control assays:

Absence of Nuclease Activity: 1  $\mu$ g of DNA / EcoR I digest was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 0.7% agarose gel.



#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2650170501730	MBD1	Lambda DNA/ <i>EcoR</i> I Digest, 50 μg, (100 loads)

# Lambda DNA/ Hind III Digest:

#### Description:

DNA was purified by cesium chloride block gradient, digested to completion with *Hind* III, phenol extracted and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. DNA / *Hind* III digest consists of 8 double stranded DNA fragments of 23130, 9416, 6557, 4361, 2322,2027, 564, and 125 base pairs.

#### Note:

- This DNA / *Hind* III digest is suitable for use as molecular weight standards for agarose gel electrophoresis.
- Heating the digest at 65oC for 5 mins and rapid cooling on ice will ensure the presence of the 4361 base pair fragments.
- Clear resolved bands are visualized on loading 1.0  $\mu g$  of DNA / Hind III digest on a 0.7 % agarose gel.
- Cannot be used for ligation experiments.
- Shipped with 6X gel loading buffer

#### Store: -20C

To avoid frequent freeze-thaw cycles, store as aliquots at -20° C.

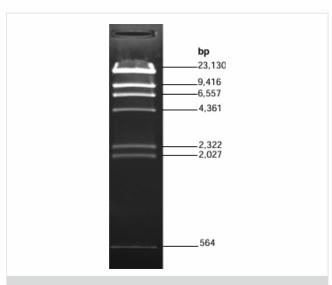


#### Related product: - RMBD2: >

Ready to use Marker is supplied at a concentration of 100 µg/ml premixed with gelloading buffer.

#### Quality control assays:

Absence of Nuclease Activity: 1  $\mu g$  of DNA / Hind III digest was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 0.7% agarose gel.



 $\mu g$  of  $\,$  DNA/ Hind III digest visualized by ethidium bromide staining on a 0.7% agarose gel

## **Ordering Information**

Cat. No	PI No.	Product Description
2660270501730	RMBD2	Lambda DNA/Hind III Digest, Ready To Use, 50 µg, (100 loads)
2650270501730	MBD2	Lambda DNA/Hind III Digest, 50 μg, (100 loads)

# Lambda DNA/EcoR I / HindIII Double Digest

## Description:

DNA was purified by cesium chloride gradient, digested to completion with *EcoR* I and Hind III,

phenol extracted and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. DNA / EcoR I -Hind III double digest consists of 13 double stranded DNA fragments of 21226, 5148, 4973, 4268, 3530, 2027, 1904, 1584, 1375, 947, 831, 564 and 125 base pairs.

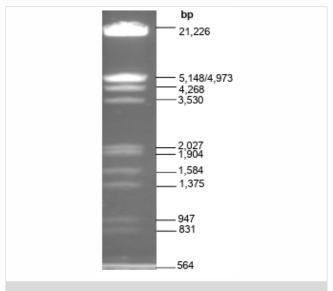
DNA was purified by cesium chloride gradient, digested to completion with *EcoR* I and Hind III, phenol extracted and supplied in 10 mM Tris-HCl (pH 8.0) and 10 mM EDTA. DNA / *EcoR* I - *Hind* III double digest consists of 13 double stranded DNA fragments of 21226, 5148, 4973, 4268, 3530, 2027, 1904, 1584, 1375, 947, 831, 564 and 125 base pairs.

#### Note:

- The DNA / EcoR I -Hind III double digest is suitable for use as molecular weight standards for agarose gel electrophoresis.
- Heating the digest at 65oC for 5 mins and rapid cooling on ice will ensure the presence of the 3530 base pair fragment.
- As the fragments are present in molar ratio, the smaller fragments will be visible only when >1 µg of marker is loaded on gel.
- Cannot be used for ligation experiments.
- Shipped with 6X gel loading buffer.
- To avoid frequent freeze-thaw cycles, store as aliquots at -20° C.

#### Quality control assays:

Absence of Nuclease Activity:  $1 \mu g$  of DNA / EcoR I - Hind III double digest was incubated with Genei Buffer for 16 hours at 37°C. Sharp unaltered band pattern was seen without smear on a 0.7% agarose gel.



 $1~\mu$  g of  $\,$  DNA/ Eco RI Hind III double digest visualized by ethidium bromide staining on a 0.7% agarose gel.



#### Related product: - RMBD3: >

Ready to use Marker is supplied at a concentration of 100  $\mu$ g/ml premixed with gel loading buffer.

#### **Ordering Information**

Cat. No	PI No.	Product Description
2660370501730	RMBD3	Lambda DNA/EcoRI- Hind III Double Digest, Ready To Use, 50 µg, (100 loads)
2650370501730	MBD3	Lambda DNA/EcoR I / Hind III Double Digest, 50 µg, (100 loads)

# PRODUCTS FOR RNA ELECTROPHORESIS

# **RNA Gel Loading Buffer**

## Description:

RNA gel loading buffer is used to load RNA sample on a formaldehyde agarose gel. It consists of two tracking dyes, Bromophenol blue that runs equivalent to 300 bp linear double stranded DNA and Xylene cyanol that has mobility equivalent to 4kb linear double stranded DNA molecule. This mobility does not change in agarose gel concentration ranging from 0.5 - 1.4%.

#### Note:

- Buffer is supplied at 5X concentration.
- Working concentration is 1X. Z

## Quality control assays:

- Absence of nuclease activity: 1 μg of EcoR I digest incubated with 1X RNA gel loading buffer, at 37° C for 16 hrs in 50 μl reaction volume, showed sharp unaltered pattern on 1% agarose gel.
- Absence of nickase activity: 1 μg supercoiled plasmid DNA incubated with 1X RNA gel loading buffer, at 37° C for 16 hrs in 50 μl reaction volume, showed unaltered pattern without nicking on 1% agarose gel.

 Absence of RNase activity: 1 μg of total RNA incubated for 4 hours at 37°C with 1X RNA gel loading buffer, in 20 μl reaction volume, showed no degradation of RNA on 2% agarose gel

#### Storage: 4°C

#### Applications:

RNA Gel Electrophoresis: RNA sample buffer is often used to denature RNA samples before loading them onto a formaldehyde gel for electrophoresis. This allows for the separation of RNA molecules based on size.

Northern Blotting: Before transfer to a membrane for Northern blotting, RNA samples are typically treated with RNA sample buffer to denature the RNA and prevent secondary structure formation. This ensures accurate sizing and detection of RNA species.

#### References:

Staining Nucleic Acids Michael R. Green and Joseph Sambrook

## **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
2606100011730)	FC61	5X RNA Gel Loading Buffer (For Electrophoresis), 4 x 0.25 ml

# RNA Gel Electrophoresis Kit

## Description:

RNA electrophoresis is used for RNA detection, quantification, separation by size, and quality assessment; since RNA molecules are negatively charged, they migrate toward the anode in the presence of electric current. The overall quality of an RNA preparation may be assessed by electrophoresing it on a denaturing agarose gel.

A denaturing gel system is required because most RNA form extensive secondary structure via intra molecular base pairing, and this prevents it from migrating strictly according to its size. A positive

# GeNei TM

control RNA can be loaded along with the samples so that unusual results can be attributed to a problem with the gel or a problem with the RNA under analysis. The integrity and size distribution of total RNA can be checked by denaturing agarose gel electrophoresis and ethidium bromide. The respective ribosomal bands should appear as sharp bands on the stained gel. 28s ribosomal RNA bands should be present with an intensity approximately twice that of the 18s rRNA band. If the ribosomal bands in a given lane are not sharp, but appear as a smear of smaller sized RNAs, it is likely that the RNA sample suffered major degradation during preparation.

#### Kit Contents:

- RNA Sample Buffer
- RNA Gel Loading Buffer
- Agarose
- 10X MOPS buffer (Powder blend)
- Formaldehyde

#### References:

- Sambrook, J. et. Al., Molecular cloning: A Laboratory Manual, Cold Spring Harbor Laboratory
- Ogden, R.C. and Adams, D.A, Electrophoresis in agarose and acrylamide gels. Meth. Enzymol. 152, 61-87, (1987)

## **Ordering Information**

Cat. No	PI No.	Product Description
2602400011730	EGK7	RNA Gel Electrophoresis Kit, 10 gels of 50ml

# FINE CHEMICALS, REAGENTS & BUFFERS

#### **IPTG**

#### Description:

Isopropyl  $\beta$ -D-Thiogalactopyranoside (IPTG) serves as a potent inducer of gene expression in bacterial systems, playing a crucial role in regulating the transcription of target genes. It mimics the structure of lactose and acts as an analog that binds to the lac repressor protein. This binding prevents the repression of the lac operon, allowing for the expression of genes under its control.

IPTG is a fundamental tool in molecular biology research, providing researchers with a controlled and inducible system for studying gene function, protein expression, and cellular processes.

Mol Wt.: 238.3, Purity: 99.7%

Storage: -20°C

#### Applications: >

- IPTG has been utilized in studies involving Lactobacillus reuteri GroEL and its mutants. The induction of protein expression in this context highlights its versatility in various bacterial systems.
- IPTG is widely employed in Escherichia coli for the induction of protein expression. This application underscores its significance in recombinant protein production and molecular biology studies using E. coli as a model organism.

#### References:

- https://pubmed.ncbi.nlm.nih.gov/33782752/
- https://pubmed.ncbi.nlm.nih.gov/35247611/

Cat. No	PI No.	Product Description
3600185001730	FC1S	IPTG, 500 mg
3600100011730	FC1L	IPTG, 1000 mg
3600100101730	FC1B	IPTG, 10 g

# GeNei TM

control RNA can be loaded along with the samples so that unusual results can be attributed to a problem with the gel or a problem with the RNA under analysis. The integrity and size distribution of total RNA can be checked by denaturing agarose gel electrophoresis and ethidium bromide. The respective ribosomal bands should appear as sharp bands on the stained gel. 28s ribosomal RNA bands should be present with an intensity approximately twice that of the 18s rRNA band. If the ribosomal bands in a given lane are not sharp, but appear as a smear of smaller sized RNAs, it is likely that the RNA sample suffered major degradation during preparation.

#### Kit Contents:

- RNA Sample Buffer
- RNA Gel Loading Buffer
- Agarose
- 10X MOPS buffer (Powder blend)
- Formaldehyde

#### References:

- Sambrook, J. et. Al., Molecular cloning: A Laboratory Manual, Cold Spring Harbor Laboratory
- Ogden, R.C. and Adams, D.A, Electrophoresis in agarose and acrylamide gels. Meth. Enzymol. 152, 61-87, (1987)

## **Ordering Information**

Cat. No	PI No.	Product Description
2602400011730	EGK7	RNA Gel Electrophoresis Kit, 10 gels of 50ml

# FINE CHEMICALS, REAGENTS & BUFFERS

#### **IPTG**

#### Description:

Isopropyl  $\beta$ -D-Thiogalactopyranoside (IPTG) serves as a potent inducer of gene expression in bacterial systems, playing a crucial role in regulating the transcription of target genes. It mimics the structure of lactose and acts as an analog that binds to the lac repressor protein. This binding prevents the repression of the lac operon, allowing for the expression of genes under its control.

IPTG is a fundamental tool in molecular biology research, providing researchers with a controlled and inducible system for studying gene function, protein expression, and cellular processes.

Mol Wt.: 238.3, Purity: 99.7%

Storage: -20°C

#### Applications: >

- IPTG has been utilized in studies involving Lactobacillus reuteri GroEL and its mutants. The induction of protein expression in this context highlights its versatility in various bacterial systems.
- IPTG is widely employed in Escherichia coli for the induction of protein expression. This application underscores its significance in recombinant protein production and molecular biology studies using E. coli as a model organism.

#### References:

- https://pubmed.ncbi.nlm.nih.gov/33782752/
- https://pubmed.ncbi.nlm.nih.gov/35247611/

Cat. No	PI No.	Product Description
3600185001730	FC1S	IPTG, 500 mg
3600100011730	FC1L	IPTG, 1000 mg
3600100101730	FC1B	IPTG, 10 g

#### X-Gal

## Description:

X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside), serves as an inert chromogenic substrate specifically for beta-galactosidase, a commonly used reporter gene in molecular biology. The enzyme hydrolyzes X-Gal, resulting in the formation of a blue precipitate. Induction of the lacZ gene with IPTG (Isopropyl  $\beta$ -D-Thiogalactopyranoside) leads to the synthesis of beta-galactosidase. In the presence of X-Gal, the hydrolysis of the substrate results in blue colonies. This phenomenon is widely used in blue-white selection assays.

Mol. wt.: 408.6., Purity by TLC >99%.

Storage: -20°C Light sensitive store in dark place

#### Applications: >

- Blue-White Screening in Cloning: X-Gal is often used in blue-white screening of bacterial colonies transformed with plasmids containing lacZ (β-galactosidase) reporter genes. In the presence of X-Gal, colonies expressing lacZ turn blue, aiding in the identification of recombinant (white) colonies from non-recombinant (blue) colonies.
- Identification of LacZ Reporter Gene Expression: X-Gal is used to identify the expression of lacZ reporter genes in various experimental systems. This can include both prokaryotic and eukaryotic systems where lacZ has been incorporated as a reporter for gene expression.
- Analysis of Promoter Activity: X-Gal is employed to assess the activity of promoters.
- Visualization of Recombinant Viruses: X-Gal is used in virology to visualize the expression of lacZ reporter genes in recombinant viruses. This aids in assessing the efficiency of virus-mediated gene transfer.

## Applications: >

 RNA Gel Electrophoresis: RNA sample buffer is often used to denature RNA samples before loading them onto a formaldehyde gel for electrophoresis. This allows for the separation of RNA molecules based on size.

 Northern Blotting: Before transfer to a membrane for Northern blotting, RNA samples are typically treated with RNA sample buffer to denature the RNA and prevent secondary structure formation. This ensures accurate sizing and detection of RNA species.

#### References: >

- https://pubmed.ncbi.nlm.nih.gov/31622532/
- https://pubmed.ncbi.nlm.nih.gov/25308773/
- https://pubmed.ncbi.nlm.nih.gov/31622532/

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
3600281001730	FC5L	X-Gal, 100 mg
3600200011730	FC5B	X-Gal, 1 g

# DTT (1,4-Dithiothreitol)

#### Description: >

DTT maintains monothiols (single thiol groups) in a reduced state. By preventing the formation of disulfide linkages, DTT helps keep individual thiol groups in their reduced, active form.

One of the key functions of DTT is its ability to quantitatively reduce disulfide bonds present in proteins. Disulfide linkages can form between cysteine residues, influencing the tertiary and quaternary structure of proteins. DTT breaks these disulfide bridges, restoring the native conformation.

DTT can restore the activity of proteins that have undergone oxidative damage. Its ability to reduce disulfide bonds and reverse oxidation-induced changes contributes to the recovery of protein function.

DTT finds extensive use in molecular biology techniques, such as protein extraction, purification, and manipulation. It is an essential component in maintaining the redox state of thiol-containing proteins



#### References:

https://pubmed.ncbi.nlm.nih.gov/23298582/

#### **Ordering Information**

Cat. No	PI No.	Product Description
3601000021730	FC22S	DTT (1,4-Dithiothreitol), 2 g
3601000101730	FC22L	DTT (1,4-Dithiothreitol), 10 g
3601000501730	FC22B	DTT (1,4-Dithiothreitol), 50 g

## **Ficoll**

#### Description:

Ficoll is a neutral, hydrophilic polysaccharide, making it suitable for use in biological solutions without causing significant chemical interactions. It has a high molecular mass, contributing to its ability to form stable density gradients in solutions.

DNase, RNase: None detected.

Storage: Room temperature.

## Applications: >

Ficoll is a crucial component of Ficoll-Paque, a density gradient medium widely used in laboratories for the separation of blood components.

Ficoll density gradient centrifugation is also used for the isolation of cells from other tissues and cell cultures.

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
3600800101730	FC39	Ficoll, 10 gms

#### Tris-Base

#### Description:

Tris(hydroxymethyl)aminomethane, commonly known as TRIS or Tris base, is a widely used buffer in biochemistry and molecular biology due to its properties as a weak base.

Purity: 99.5%,

Molecular Weight: 121.14

Storage: Room Temperature

#### Applications:

- TRIS is commonly used as a buffering agent to maintain a stable pH in various biochemical and molecular biology assays, such as enzyme assays, nucleic acid isolation, and protein purification.
- TRIS is frequently used as a component of electrophoresis buffers for DNA, RNA, and protein gel electrophoresis.
- TRIS is a common constituent in the running buffer for polyacrylamide gel electrophoresis, especially for the separation of proteins at neutral pH.
- TRIS is used in the formulation of cell culture media to control and maintain the pH of the media, creating an optimal environment for cell growth.
- TRIS is employed in various steps of nucleic acid extraction and purification protocols. It helps maintain the pH of the buffers.

Cat. No	PI No.	<b>Product Description</b>
3600701001730	FC30S	Tris-Base, (Molecular Biology Grade), 100 g
3600710001730	FC30L	Tris-Base, (Molecular Biology Grade), 1 Kg
3600750001730	FC30B	Tris-Base, (Molecular Biology Grade), 5 Kg

#### Tween<sub>8</sub>-20

#### Description:

Tween®20 is a polyoxyethylene sorbitol ester that belongs to the polysorbate family. Tween®20 is a nonionic detergent classified under the polysorbate family. It is characterized by its polyoxyethylene sorbitol ester structure. The molecular weight is approximately 1,225 daltons, with a composition of 20 ethylene oxide units, 1 sorbitol, and 1 lauric acid as the primary fatty acid. The ethylene oxide subunits contribute to the hydrophilic character of Tween®20, making it soluble in aqueous solutions. At the same time, the lauryl group imparts a hydrophobic element. This balanced hydrophilichydrophobic nature is advantageous in solubilizing hydrophobic molecules in aqueous environments.

pH Stability: Stable pH 6.5 - 7.5.

Storage: Room temperature. Protect from light.

#### Applications: >

- It is widely used as a solubilizing agent, facilitating the dispersion of hydrophobic substances (such as lipids, proteins, or small molecules) in water-based solutions.
- It is utilized as a blocking agent in immunoassays to prevent nonspecific binding.
- It serves as a washing agent to remove unbound molecules, contributing to the specificity of the assav.
- Tween®20 is often included in buffers or solutions to enhance the permeability of cell membranes, allowing antibodies to access intracellular targets more effectively.

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
3601181001730	FC31	Tween® -20, 100 ml

# **Guanidine Hydrochloride**

#### Description:

Guanidine hydrochloride ( $NH_2C(=NH)NH_2 \cdot HCl$ ) is a Strong chaotropic agent useful for the denaturation and subsequent refolding of proteins. This strong denaturant can solubilize insoluble or denatured proteins such as inclusion bodies.

Molecular Weight: 95.53

**Purity:** > 98%

Storage: Room Temperature

#### Applications: >

It is used as a component of the extraction buffer for the extraction of proteoglycans

It is used in extraction during protein fractionation of ATDC5 cell lines

It is used as a chemical additive to study its effective absorbance spectra in structural analysis

It is used in RNA isolation to dissociate nucleoproteins and inhibit RNase

It plays a role in inhibiting heat shock protein 104 (Hsp104) adenosine triphosphatase (ATPase) activity in vivo.

It is a potent denaturant and inactivator of several enzymes and proteins. GuHCl can inactivate aminoacylase and papain

#### References:

- https://pubmed.ncbi.nlm.nih.gov/31622532/
- https://pubmed.ncbi.nlm.nih.gov/25308773/
- https://pubmed.ncbi.nlm.nih.gov/31622532/

Cat. No	PI No.	<b>Product Description</b>
3600800101730	FC39	Ficoll, 10 gms



#### **CTAB Powder**

#### Description:

Hexadecyltrimethylammonium Bromide (CTAB) is a cationic surfactant belonging to the quaternary ammonium salt family. Also known as cetyltrimethylammonium bromide, it consists of a hydrophilic quaternary ammonium cation (head) and a hydrophobic hexadecyl chain (tail). The molecular structure of CTAB features a positively charged ammonium group with three methyl groups attached to a long hydrophobic carbon chain containing 16 carbon atoms (hexadecyl).

Molecular Formula: C<sub>19</sub>H<sub>42</sub>BrN

Molecular Weight: 364.5

Purity: 99%

Storage: Room Temperature

#### Applications: >

In molecular biology, CTAB is employed in DNA and RNA extraction methods, such as the CTAB method for plant genomic DNA extraction. It helps in the removal of proteins, polysaccharides, and other contaminants.

CTAB plays a crucial role in the synthesis of nanoparticles, particularly gold and silver nanoparticles. It acts as a stabilizing agent, preventing the agglomeration of particles during formation.

Due to its cationic nature, CTAB exhibits antimicrobial properties. It is incorporated into products like shampoos and mouthwashes for its ability to disrupt bacterial cell membranes.

CTAB is soluble in water, forming micelles at concentrations above its Critical Micelle Concentration (CMC). These micelles are essential in various processes, such as solubilization, emulsification, and stabilizing colloidal systems

#### References: >

https://pubmed.ncbi.nlm.nih.gov/2329858

#### **Ordering Information**

Cat. No	PI No.	<b>Product Description</b>
3600600501730	FC36	Guanidine Thiocyanate, 50g

# PRODUCTS FOR NUCLEIC ACID ELECTROPHORESIS

## **High Resolution Agarose**

High Resolution Agarose is white fine homogeneous powder used in gel electrophoresis.

• Gel Strength (g/cm2): 448g/cm2

Gelling temp: 32.5°C

Melting temp:75°C

• Moisture: 5.6%

• EEO(-Mr):0.05

Store: Room Temperature

Note: Cool the solution to  $50^\circ$ C to  $60^\circ$ C prior to casting. Once the gel is cast, allow the molten agarose to cool and gel at room temperature.

#### Performance Test:

Gel electrophoresis of 20 bp DNA Ruler on 2% gel was performed, expected band patterns were visualized by ethidium bromide stain.

## References: >

Jeppsson JO, Laurell CB, Franzén B (April 1979). "Agarose gel electrophoresis". Clinical Chemistry. 25 (4): 629–38.

Akari C (1956). "Structure of the agarose component of agar-agar". Bulletin of the Chemical Society of Japan

Cat. No	PI No.	Product Description
2600301001730	HAG4	High Resolution Agarose, 100g

# AGAROSE BUFFERS FOR ELECTROPHORESIS

#### **50X TAE**

#### Description:

In 1946, Gomori (1946) suggested that organic polyamines could be used to control pH in the range 6.5–9.7. One of the three compounds he investigated was Tris(2-amino-2-hydroxymethyl-1,3-propanediol), which had been first described in 1897 by Piloty and Ruff. One of Tris' first commercial successes, which received wide attention, was the reduction of mortality during handling and hauling of fish. Tris [Tris(hydroxymethyl)aminomethane] has a very high buffering capacity, is highly soluble in water, and is inert in a wide variety of enzymatic reactions. However, Tris also has a number of deficiencies. The pKa of Tris is pH 8.0 (at 20°C), which means that its buffering capacity is very low at pHs 9.0.

TAE (Tris-Acetate-EDTA) buffer stands as a widely utilized buffer solution in molecular biology, finding prominence, especially in the realm of agarose gel electrophoresis. This buffer serves a pivotal role in providing the necessary ionic environment for the separation of nucleic acids, such as DNA or RNA, during electrophoresis. Use of weak acids provide the proper ion concentration and maintain appropriate pH while nucleic acids move through the agarose matrix.

## Applications: >

- DNA Fragmentation and Separation: TAE is crucial for separating DNA fragments in agarose gels according to their molecular size. This is a fundamental step in various molecular biology applications, including DNA fingerprinting, DNA profiling, and the analysis of PCR products.
- DNA Gel Extraction: TAE can be used in procedures where specific DNA fragments are excised from agarose gels for further analysis or downstream applications. This is common in techniques like gel extraction or PCR purification.

**Storage:** Room Temperature, for long term storage at 4°C

#### References:

- Brody, J.R., Kern, S.E. (2004) History and principles of conductive media for standard DNA electrophoresis. Anal Biochem. 333(1):1-13. doi:10.1016/j.ab.2004.05.054 PMID 15351274 PDF
- Sambrook, Fritsch, and Maniatis (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, volume 3, apendices B.11 and B.23 ISBN 0-87969-309-6

#### **Ordering Information**

Cat. No	PI No.	Product Description
2601182001730	FC14	50X TAE, 200 ml
2601110001730	FC14J	50X TAE, 1000 ml

#### **10X TBE**

TBE buffer, Tris-Borate-EDTA, is a common buffer solution used in molecular biology and biochemistry for various applications, especially in nucleic acid electrophoresis. The buffer is named after its three main components: Tris, boric acid (borate), and EDTA. TBE is useful in the separation of short fragments lesser than 1 Kb and has a significantly higher buffering capacity, but borate involved in this buffer could potentially inhibit the activity of enzymes. Thus if the electrophoresis is followed by nucleic acid isolation from the gel and other steps (e. g. ligation)TBE is not preferred.TBE has a higher buffering capacity than TAE, so it works better for a longer run. The effective range of Tris is 7-9. The effective range of boric acid is 8-10,the overlap of buffering properties of both is 8-9, thus mostly used pH 8.3 was found to be optimal.

#### Applications:

**Agarose Gel Electrophoresis**: TBE is commonly used as a running buffer for agarose gel electrophoresis, a technique used to separate nucleic acids (DNA or RNA) based on size.

**Polyacrylamide Gel Electrophoresis (PAGE):** TBE is also used in polyacrylamide gel electrophoresis for higher resolution separation of nucleic acids.

**Nucleic Acid Electrophoresis:** TBE is suitable for the electrophoretic separation of DNA and RNA fragments. It provides the necessary ions for electrical current and maintains a stable pH.



**Southern Blotting and Northern Blotting**: TBE is often used in the preparation of agarose gels for these blotting techniques, where nucleic acids are transferred to a membrane for further analysis.

**Buffer for Enzymatic Reactions:** TBE can be used as a buffer for various enzymatic reactions involving nucleic acids.

Storage: Room Temperature

#### **Ordering Information**

Cat. No	PI No.	Product Description
2601282001730	Fc40	10X TBE, 200 ml
2601285001730	FC40L	10X TBE, 500 ml
2601210001730	FC40J	10X TBE, 1000 ml

#### **MOPS Buffer**

MOPS is a zwitterionic biological buffer with a useful buffering range of 6.5-7.9, specially tested and certified to be free of DNase, RNase, and protease activity for molecular biology application.

Molecular Weight: 209.3 Formula: C<sub>7</sub>H<sub>15</sub>NO<sub>4</sub>S CAS No.: 1132-61-2

Useful pH Range: 6.5-7.9
Solubility: Soluble in water
Storage: Room Temperature

The MOPS buffer solution, formulated at a 10X concentration with a pH of 7.0, serves as a low ionic strength buffer specifically designed for the electrophoretic separation of formaldehydedenatured RNA. This buffer is carefully crafted to provide optimal conditions for the migration of RNA molecules during electrophoresis, ensuring efficient and accurate separation based on size. The low ionic strength of the MOPS buffer is particularly advantageous for maintaining the denatured state of RNA, allowing for precise analysis and resolution of RNA fragments in various molecular biology applications.

#### Applications:

RNA Gel Electrophoresis: MOPS buffer is commonly used as a running buffer for agarose or polyacrylamide gel electrophoresis of RNA molecules. It helps separate RNA fragments based on size.

**Northern Blotting:** MOPS buffer is used in the preparation of agarose or polyacrylamide gels for northern blotting. In this technique, RNA molecules separated by gel electrophoresis are transferred to a membrane and probed for specific sequences.

**RNA Isolation:** MOPS buffer is sometimes used in procedures involving the isolation of RNA. It can be employed in various RNA extraction protocols.

cDNA Synthesis (Reverse Transcription): MOPS buffer may be used in reverse transcription reactions, where RNA is converted into complementary DNA (cDNA) using reverse transcriptase.

**In Vitro Transcription Reactions**: MOPS buffer can be used in the preparation of buffers for in vitro transcription reactions, where RNA is synthesized from a DNA template using RNA polymerase.

Protein Electrophoresis: While MOPS buffer is more commonly associated with RNA applications, it can also be used in certain protein electrophoresis applications, particularly in native gel electrophoresis.

**Buffer for Enzymatic Reactions:** MOPS buffer can serve as a suitable buffer in various enzymatic reactions involving nucleic acids, including certain restriction enzyme digestions.

**Quality Control in RNA Studies:** MOPS buffer may be used in the assessment of RNA quality, especially in situations where maintaining RNA stability is crucial

**Native Gel Electrophoresis:** MOPS buffer can be employed in native gel electrophoresis, a technique used to separate biomolecules under conditions that preserve their native structures.

Cat. No	PI No.	<b>Product Description</b>
3601805001730	FC96M	MOPS, Molecular Biology Grade, 500 g
3601905001730	FC97M	MOPS Sodium Salt, Molecular Biology Grade, 500 g



#### **DNA GEL LOADING BUFFERS**

#### Applications: >

- The inclusion of a densifying agent in DNA Gel Loading buffers serves a pivotal role in ensuring that the sample efficiently settles at the bottom of the well during gel loading. Additionally, the presence of a metal ion binder in the buffer plays a crucial role by binding divalent metal ions, thereby inhibiting nuclease action and providing protection to the DNA.
- Moreover, the incorporation of 6X dyes in the buffer facilitates the visual tracking of DNA migration during electrophoresis. This not only aids in real-time monitoring but also eliminates any masking of the DNA bands when exposed to UV light. These dyes find versatile applications in the analysis of DNA samples, whether through Agarose or polyacrylamide gel electrophoresis, providing researchers with a reliable and efficient tool for accurate DNA characterization.

## **6X Gel Loading Buffer**

- The 6X DNA Loading Dye is tailored for the preparation of DNA markers and samples intended for agarose gel. This formulation integrates two dyes, facilitating visual tracking of DNA migration during electrophoresis. Incorporating glycerol is pivotal, promoting the formation of a distinct layer at the well's bottom for even loading of DNA ladders and samples. This ensures precise positioning, facilitating subsequent DNA band analysis.
- ◆ The buffer consists of two dyes Bromophenol Blue and Xylene cyanol FF. Bromophenol Blue migrates through agarose gels at approximately the same rate as linear ds DNA 300 bp in length whereas xylene cyanol FF migrates at approximately the same rate as linear ds DNA 4 kb in length. These relationships are not significantly affected by the concentration of agarose in the gel over the range of 0.5% 1.4%.
- 1X Gel Loading Buffer:
- · 0.04% Bromophenol Blue, 0.04% Xylene Cyanol FF, 2.5% Ficoll and 10 mM EDTA.

- Buffer supplied at 6X concentration.
- Working concentration is 0.5 1X

Storage: -20°C

# **6X Orange Gel Loading Buffer:**

The 6X Orange Gel Loading Buffer stands out as a highly beneficial dye for agarose gel electrophoresis when used at a 1X concentration. Comprising a single dye, Orange G, it offers distinctive advantages in DNA fragment analysis. Notably, the Orange G dye exhibits faster migration compared to Bromophenol blue, and its mobility aligns approximately with a 50 bp DNA fragment. This unique property facilitates efficient tracking and visualization of DNA migration during electrophoresis, particularly in the lower size range of DNA fragments.

- Buffer supplied at 6X concentration.
- Working concentration is 1X.

Storage: -20°C

## **6X Tri Gel Loading Buffer**

6X Tri gel loading buffer is used as DNA tracking dye in agarose gel electrophoresis. The buffer consists of three dyes - Bromophenol Blue, Xylene cyanol FF and OrangeG. Bromophenol Blue migrates through agarose gels at approximately the same rate as linear ds DNA 300 bp in length, xylene cyanol FF migrates at approximately the same rate as linear ds DNA 4 kb in length whereas Orange G migrates at approximately the same rate as linear ds DNA 50 bp in length. These relationships are not significantly affected by the concentration of agarose in the gel over the range of 0.5% - 1.5%.

Note: On a short run bromophenol blue and orange G interact with each other and may fluoresce in the UV light

1X Tri Gel Loading Buffer: 0.013% Bromophenol Blue, 0.01% Xylene Cyanol FF, 0.05% Orange G and 8 mM EDTA.



#### Quality control assays: >

- Absence of Nuclease Activity: 1 μg of buffered /EcoR I digest was incubated for 16 hours with 1X gel loading buffer at 37°C. Sharp unaltered banding pattern without smear was visualized on 1% agarose gel.
- Absence of Nickase Activity: 1 g of buffered supercoiled plasmid DNA when incubated with 1X Gel loading buffer at 37°C showed unaltered pattern without nicking.

Storage: -20°C

# NanoGreen DNA Elpho Buffer,400ml

NanoGreen DNA Elpho Buffer is a safe alternative to Ethidium bromide for visualization of DNA electrophoresed on agarose gel. It is a reagent used in preparation of agarose gels and as electrophoretic (Running) buffer. The purified DNA can be used for restriction digestion, ligation, amplification and cloning applications.

Storage: Room Temperature

#### Quality control assays: >

- Sensitivity test: On using 1X concentration of NanoGreen DNA Elpho Buffer, as low as 50 ng of lambda DNA was visualized.
- ◆ Performance Test: NanoGreen DNA Elpho Buffer (1X) was used to electrophorese PCR product (~1.1 kb) on agarose gel. Subsequent extraction and purification was done. Purified product thus obtained could be cloned successfully into linearized pUC-vector.

# 6X Orange G/ Cresol red DNA loading dye

6X Orange-G/Cresol Red DNA Loading Dye is used as DNA tracking dye in agarose gel electrophoresis. The dye consists of orange-G, Cresol Red sodium salt dissolved in tris buffer and glycerol. Orange-G/Cresol-Red dye migrates through the 1% Agarose gel at approximately the same rates linear 1.5 kb for cresol red 40-50 bp for orange-G. Dye migration

rate on different agarose gel concentration is mentioned below.

SI. no	% of agarose gel	Migration rate of Orange G
1	0.7%	100 bp
2	1.0%	50 bp
3	1.5%	20 bp
4	2.0%	<10 bp
5	3.0%	<10 bp

1X Orange-G/Cresol-Red DNA Loading Dye Composition:

- Cresol Red Sodium Salt 0.04%
- Glycerol 6.6%
- ◆ Tris 1.7mM
- ◆ Orange -G 0.04%
- ◆ EDTA 10mM

Storage: -20°C

# **6X Cresol-Red DNA Loading Dye**

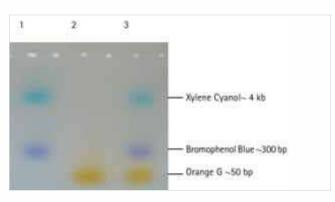
6X Cresol Red DNA Loading Dye is used as DNA tracking dye in agarose gel electrophoresis. The Gel Loading Dye consists of Cresol Red sodium salt dissolved in tris buffer and glycerol. Cresol Red dye migrates through the agarose gels at approximately 1.5 kb. Dye migration rate on different agarose gel concentration is mentioned below

SI, no % of agarose ge		Migration rate of Cresol Red		
1	0.7%	3000 bp		
2	1.0%	1500 bp		
3	1.5%	900 bp		
4	2.0%	300 bp		
5	3.0%	>100 bp		

1X Cresol Red DNA Loading Dye Composition:

- Cresol Red Sodium Salt 0.04%
- Glycerol 6.6%
- Tris − 1.7mM
- ◆ EDTA 10mM

Storage: -20°C



Lane 1 - Cat No. 612601480061730 Lane 2 - Cat No. 612601580061730 Lane 3 - Cat No. 612601680061730

- DNA Gel Electrophoresis: Gel loading buffer is commonly used to prepare DNA samples for agarose gel electrophoresis. The tracking dyes provide visual cues for the migration of DNA through the gel, and the density agents ensure that the sample sinks into the gel wells.
- RNA Gel Electrophoresis: Like DNA, gel loading buffer is used to prepare RNA samples for electrophoresis. The dyes aid in tracking RNA migration, and the density agents help load the sample into the gel.
- Size Fractionation: Gel loading buffer is essential for preparing samples when the goal is to separate molecules based on size, as in DNA, RNA, or protein electrophoresis.
- Quality Control: Gel loading buffer is used in quality control procedures to assess the integrity and size distribution of nucleic acids or proteins.
- Monitoring Electrophoresis Run: The tracking dyes in the gel loading buffer allow researchers to monitor the progress of the electrophoresis run, ensuring that samples are migrating appropriately through the gel.
- Agarose Gel Extraction: After electrophoresis, samples in gel loading buffer can be purified or extracted for further downstream applications, such as DNA fragment isolation.
- Enzyme Digestion Quality Control: Gel loading buffer is used to assess the success of enzyme digestion reactions by checking the size distribution of DNA fragments after digestion.

#### References:

 Products: Color Markers for Electrophoresis". Science. 277 (5328): 979. 1997-08-15

https://pubchem.ncbi.nlm.nih.gov/summary/summary.

#### **Ordering Information**

Cat. No	PI No.	Product Description	
2601480061730	FC15	6X Gel Loading Buffer, 6 ml	
2601580061730	FC48	6X Orange Gel Loading Buffer, 6 ml	
2602584001730	FC101M	NanoGreen DNA Elpho Buffer,400ml	
2601680061730	FC63	6X Tri Gel Loading Buffer, 6 ml	
2602680061730	FC75	6X Orange G/ Cresol red DNA loading dye, 6X1 ml	
2602580061730	FC74	6X Cresol-Red DNA Loading Dye, 6 x 1 ml	

# Nucleic Acid Agarose Gel Electrophoresis Kit

Nucleic acid agarose gel electrophoresis is a widely used technique in molecular biology for the separation and analysis of DNA and RNA molecules. The gel matrix allows the molecules to migrate through it based on size, and this separation is visualized by staining the nucleic acids with a fluorescent dye.

## Applications: >

- Agarose gel electrophoresis is commonly used to determine the size of DNA or RNA fragments. By comparing the migration of unknown fragments to that of a known molecular weight marker, researchers can estimate the size of the sample molecules.
- Agarose gel electrophoresis is used to assess the purity of DNA or RNA samples. Contaminants, such as proteins or other nucleic acids, can be visualized as additional bands or smearing on the gel.
- Agarose gel electrophoresis is routinely used to analyze PCR products. It allows researchers to confirm the presence, size, and purity of the amplified DNA fragments.
- Agarose gel electrophoresis is employed to confirm the presence and size of plasmid DNA after cloning procedures. It helps researchers verify the success of plasmid preparation and the insertion of desired DNA fragments.



#### **Ordering Information**

Cat. No	PI No.	Product Description
2600480101730	FC38	Ethidium Bromide, 10 mg
2602300011730	EGK6	Nucleic Acid Agarose Gel Electrophoresis Kit (consumables) (with Ethidium Bromide), 25 gels of 100ml

## Silver staining-DNA

- Silver staining is a highly sensitive method for staining DNA, an excellent method for characterization of DNA in polyacrylamide gels. This method for visualizing bands of DNA separated on polyacrylamide gels is more sensitive than the Ethidium bromide method for double stranded DNA. It can detect nano gram quantities of DNA and can also detect single stranded DNA or RNA with equal sensitivity. Silver staining of gels creates a permanent record of the gels and visualization does not need any special equipment. The staining is a multi-step procedure requiring high quality reagents. Nucleic acids reduce silver cations to insoluble silver which deposits in the gel around the DNA bands. The bands appear dark brown or black and the reaction is stopped by altering the pH.
- The silver staining process consists of the following steps: Fixing, sensitization, silver impregnation, development, stopping, and gel preservation with intermittent water washes.
- Fixing makes the macromolecules in the gel insoluble and prevents them from diffusing out of the gel during subsequent staining steps.
- Sensitization The gel is treated with reagents that render the DNA more reactive towards silver and accelerate the subsequent reduction of silver ion. Excess sensitization reagent result in a high level of background staining.
- Silver impregnation Gel is treated with silver nitrate under mildly acidic conditions thus preventing silver ion from being reduced to metallic silver.

- Development The development solution contains formaldehyde, which reduces silver ion to metallic silver only at high pH. Hence, sodium carbonate is included to render the development solution alkaline.
- Stopping and gel preservation The stopping solution prevents further reduction of silver ion.
   The preservation can be done using glycerol, which prevents the gel from cracking during drying.
- DNA Silver Staining Kit is highly sensitive, fast, reproducible, and non-radioactive method of staining DNA in polyacrylamide gels.

#### Features:

- Ready-to-use solutions for maximum ease of use and reliability.
- The kit is supplied with a suitable DNA Molecular Weight Marker
- Low background
- Non-radioactive
- Fast detection- nucleic acid bands are visualized within 2 hours.
- Reproducible
- Highly sensitive, stains up to100pg of nucleic acid, which is 50 times more sensitive than Ethidium Bromide Method

## Applications: >

◆ Sensitive detection of DNA electrophoresed on native polyacrylamide gels

#### References:

- Berry, M. J. and Samuel, C. E., (1982) "Detection of Subnanogram Amounts of RNA in Polyacrylamide Gels in Presence and Absence of Protein by Staining with Silver," Anal. Biochem., 124, 180.
- McNeilage, L. J. and Whittingham, S., (1984) "Use of Bio Rad Silver Stain to Identify Gel Purified RNA Components of Small Nuclear Ribonucleoprotein Antigens," J. Immunol. Methods, 66, 253
- Goldman, D. and Merril, C. R., (1982) "Silver Staining of DNA in Polyacrylamide Gels: Linearity and Effect of Fragment Size," Electrophoresis, 3, 24
- Beidler, J. L., et al., (1982) "Ultrasensitive Staining of Nucleic Acids with Silver," Anal. Biochem., 126, 374

Cat. No	PI No.	<b>Product Description</b>
2602100011730	KT86	DNA Silver staining Kit, 10 gels (15 x 18 cm)