TOOLS FOR PROTEOMIC RESEARCH

TOOLS FOR PROTEIN RESEARCH

Kits for Protein Purification & Analysis

GST - Fusion Protein Purification Kit
His - Tag Fusion Protein Purification Kit (IDA Based)

Protein Estimation Kits

Protein Estimation Kit by Lowry's Method
Protein Estimation Kit by Biuret Method
Protein Estimation Kit by BCA Method
Protein Estimation Kit by Bradford Macro Method
Protein Estimation Kit by Bradford Micro Method
Native PAGE reagent Kit with Marker
Silver Staining Kit with Marker (Protein)
Western Blot Development Kits

Electrophoresis Chemicals

Acrylamide
Bis-acrylamide
Acrylamide/bis-acrylamide solution
Ammonium persulphate
TEMED

BUFFERS, STAINING & FIXING SOLUTIONS FOR PAGE

Kits for Protein Purification & Analysis

Tris-SDS, pH 8.8
Tris-SDS, pH 6.8
Tris-glycine-SDS buffer
Sample Loading buffer for SDS PAGE
10X Tris Tricine SDS Buffer
Coomassie Gel Stainer
Coomassie Gel Destainer

10 X PBS, pH 7
10X ELISA Blocking buffer (BSA)
ELISA Blocking Buffer (Casein)
10X ELISA Blocking Buffer (Gelatin)
5X Blot Transfer Buffer
Ezeeblue Gel Stainer

FOR WESTERN BLOTTING:

Ready to use Ponceau-S
Affinity Media & Columns Selection guide
for affinity matrices by their application
Protein A-CL Agarose

POLYCLONAL ANTIBODIES

Antibodies to Whole Serum Antibodies to Immunoglobulins (Secondary Antibodies)

CONJUGATES OF SECONDARY ANTIBODIES

Introduction
Enzyme Conjugates
Flurochrome Conjugates
Biotin Conjugates
Gold Conjugates

Purification of His Tag fusion protein (NTA based) Kit

Description :

The development of expression vectors designed to provide various fusion tags to the expressed protein have made recombinant protein purification much easier. The expression vectors that provide polyhistidine tags (6X His) are widely used for rapid purification of the expressed protein of interest. Metal chelate or immobilized metal affinity chromatography (IMAC) is a powerful technique for isolating recombinant fusion proteins under mild conditions. A metal chelating group immobilized on a chromatographic medium, binds a multivalent metal ion (usually Cu2+, Ni2+, Zn2+, or Co2+) in a way that leaves some coordination sites free for selective interaction with proteins. Typically, 5–6 histidine residues ("tag") are added to the C- or N-terminus of a target protein using recombinant techniques. The tag specifically interacts with the chelated metal ions, thereby holding these proteins on the medium. Other components bind weakly or not at all. Elution of the fusion protein is usually performed by increasing the concentration of a competitive eluting agent, such as Imidazole. Description: His tag fusion protein purification kit is a cross linked agarose based resin pre-charged with Ni2+ions. Binding of the protein is based on the interaction between the polyhistidine tag of the recombinant protein and immobilized Ni2+ions.· Nickel is chelated on CL-Agarose through nitrilotriacetic acid which enables strong and efficient binding of target protein due to its affinity for 6X His Tag. This enables rapid purification of polyhistidine tag fusion protein from bacterial lysate. Nitrilotriacetic acid (NTA) is a tetra-dentate chelator which occupies 4 of the 6 binding sites in the coordination sphere of Ni2+ions. The remaining 2 coordination sites are usually occupied by water molecules and can be exchanged with histidine residues of the recombinant protein.

Features :

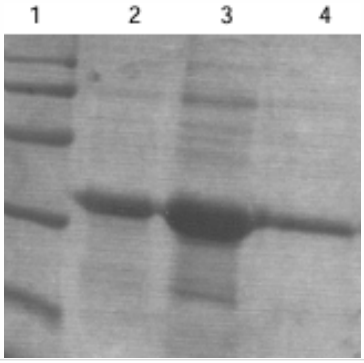
- Fast and easy method.
- Powerful method of purification
- One step purification.

Kit Contents :

- Nickel CL-Agarose column
- 2X Equilibration Buffer
- 2X Wash Buffer
- Elution Buffer

Applications :

For purification of his-tagged proteins.



Analysis of Crude lysate and His-tag protein purified using His-tag Fusion Protein Purification Kit by SDS-PAGE.

Lane 1 : Protein Molecular Weight Marker, Medium Range
Lane 2 : Purified His tag fusion protein 5µg
Lane 3 : Purified His tag fusion protein 25µg
Lane 4 : Purified His tag fusion protein 2µg

References :

- Lindner, P., et al. (1992) Purification of Native Proteins from the Cytoplasm and Periplasm of Escherichia coli Using IMAC and Histidine Tails: A Comparison of Proteins and Protocols. METHODS: A Companion to Methods in Enzymology 4: 41–56.
- Porath, J. (1992) Immobilized Metal Ion Affinity Chromatography. Protein Expression and Purification 3: 263–281.

Ordering Information:

Cat. No	PI No.	Product Description
2160900011730	KT237	Purification of His Tag fusion protein (NTA based) Kit, 4 preps

His - Tag Fusion Protein Purification Kit (IDA Based)

Description :

The development of expression vectors designed to provide various fusion tags to the expressed protein have made recombinant protein purification much easier. The expression vectors that provide polyhistidine tags (6X His) are widely used for rapid purification of the expressed protein of interest. Metal chelate or immobilized metal affinity chromatography (IMAC) is a powerful technique for isolating recombinant fusion proteins under mild conditions. A metal chelating group immobilized on a chromatographic medium, binds a multivalent metal ion (usually Cu2+, Ni2+, Zn2+, or Co2+) in a way that leaves some coordination sites free for selective interaction with proteins. Typically, 5–6 histidine residues ("tag") are added to the C- or N-terminus of a target protein using recombinant techniques. The tag specifically interacts with the chelated metal ions, thereby holding these proteins on the medium. Other components bind weakly or not at all. Elution of the fusion protein is usually performed by increasing the concentration of a competitive eluting agent, such as Imidazole. Description: His tag fusion protein purification kit is a cross linked agarose based resin pre-charged with Ni2+ions. Binding of the protein is based on the interaction between the polyhistidine tag of the recombinant protein and immobilized Ni2+ions. Nickel is chelated on CL-Agarose through nitrilotriacetic acid which enables strong and efficient binding of target protein due to its affinity for 6X His Tag. This enables rapid purification of polyhistidine tag fusion protein from bacterial lysate. Nitrilotriiacetic acid (NTA) is a tetra-dentate chelator which occupies 4 of the 6 binding sites in the coordination sphere of Ni2+ions. The remaining 2 coordination sites are usually occupied by water molecules and can be exchanged with histidine residues of the recombinant protein.

Features :

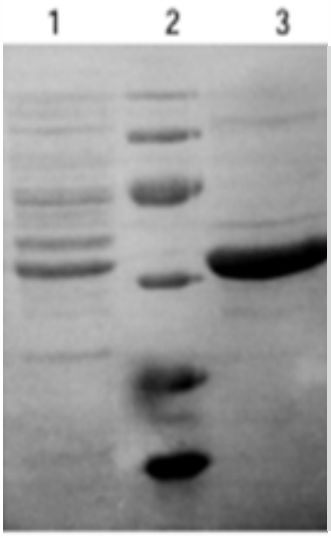
- Fast and easy method.
- Powerful method of purification
- One step purification.

Kit Contents :

- Nickel CL-Agarose column
- 5X Equilibration Buffer
- 5X Wash Buffer
- 5X Elution Buffer

Applications :

For purification of his-tagged proteins.



Analysis of Crude lysate and His-tag protein purified using His-tag Fusion Protein Purification Kit by SDS-PAGE.

Lane 1: Crude Lysate (25 µl)

Lane 2: Protein Molecular Weight Marker

Lane 3: Purified His Tag Fusion Protein

References :

- Lindner, P., et al. (1992) Purification of Native Proteins from the Cytoplasm and Periplasm of Escherichia coli Using IMAC and Histidine Tails: A Comparison of Proteins and Protocols. METHODS: A Companion to Methods in Enzymology 4: 41–56.
- Porath, J. (1992) Immobilized Metal Ion Affinity Chromatography. Protein Expression and Purification 3: 263–281.

Ordering Information:

Cat. No	PI No.	Product Description
2160700011730	KT65	His - Tag Fusion Protein Purification Kit, 5 preps

GST - Fusion Protein Purification Kit

Description :

GeNei™ The expression and purification of recombinant proteins is central to protein regulation, structure, and function studies. Most recombinant proteins are expressed as fusions with short affinity tags or small proteins, such as glutathione S-transferase (GST). The use of Glutathione S-transferase (GST) gene fusion proteins is a method for inducible, high-level protein expression and purification from bacterial cell lysates. The use of GST as a fusion tag is desirable because it can act as a chaperone to facilitate protein folding, and frequently the fusion protein can be expressed as a soluble protein rather than in inclusion bodies. Additionally, the GST fusion protein can be affinity purified facilely without denaturation or use of mild detergents. The fusion protein is captured by immobilized glutathione and impurities are washed away. The fusion protein then is eluted under mild, non-denaturing conditions using reduced glutathione. If desired, the removal of the GST affinity tag is accomplished by using a site-specific protease recognition sequence located between the GST moiety and the target protein.

GST Fusion Protein Purification Kit is designed for rapid affinity purification of glutathione S-transferase (GST) fusion protein from bacterial cell lysate. This protein binds specifically to reduced glutathione (GSH) in near-neutral, non-denaturing conditions (e.g., Tris buffer). Bound protein is easily dissociated (eluted) by competitive displacement with buffer containing free, reduced GSH (oxidized glutathione, GSSH is not effective for this purpose). When proteins of interest are expressed as fusions with GST and glutathione is immobilized to an solid support, this protein-substrate system enables affinity purification of recombinant proteins, as well as various other experiments with those proteins

Features :

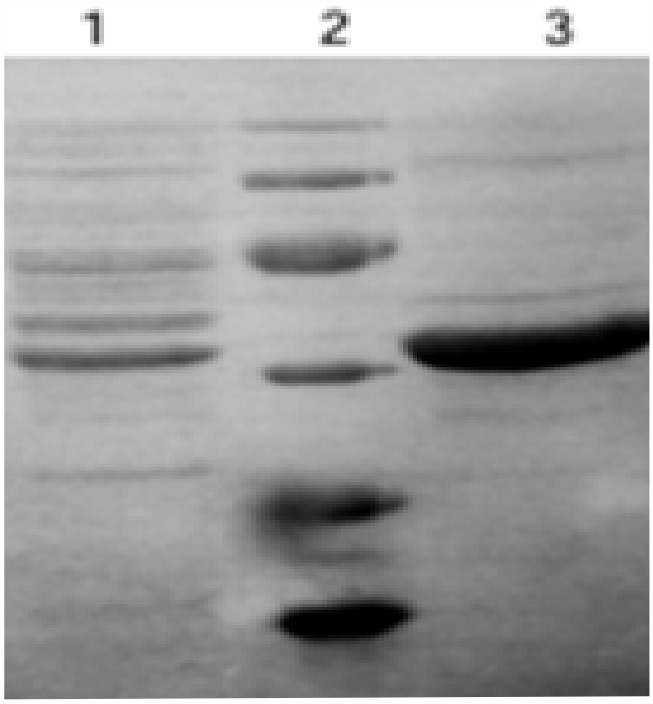
- Fast and easy method.
- Powerful method of purification
- One step purification.

Kit Contents :

- Nickel CL-Agarose column
- 5X Equilibration Buffer
- 5X Wash Buffer
- 5X Elution Buffer

Application :

For purification of his-tagged proteins.



Analysis of Crude lysate and His-tag protein purified using His-tag Fusion Protein Purification Kit by SDS-PAGE.

Lane 1: Crude Lysate (25 µl)
Lane 2: Protein Molecular Weight Marker
Lane 3: Purified His Tag Fusion Protein

References :

- Lindner, P., et al. (1992) Purification of Native Proteins from the Cytoplasm and Periplasm of Escherichia coli Using IMAC and Histidine Tails: A Comparison of Proteins and Protocols. METHODS: A Companion to Methods in Enzymology 4: 41–56.
- Porath, J. (1992) Immobilized Metal Ion Affinity Chromatography. Protein Expression and Purification 3: 263–281.

Ordering Information:

Cat. No	PI No.	Product Description
2160600011730	KT64	GST - Fusion Protein Purification Kit, 5 preps

Protein Estimation Kit by BCA Method

Description :

Protein estimation based on Bicinchoninic acid (BCA) is the most sensitive and detergent compatible method for the colorimetric detection and quantitation of total protein. BCA method is based on the principle of the reduction of Cu++ to Cu+ by protein in an alkaline medium and highly sensitive and the selective colorimetric detection of the cuprous cation (Cu+) with reagent containing Bicinchoninic acid1. The purple colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm.

Features :

- Kit contains highly sensitive and stable reagents.
- Kit provides a linear range of estimation from 10µg to 1000 µg of protein with linear increase in absorbance at 562nm.
- The rate of color development is slow and hence large number of samples can be processed simultaneously for estimation of protein.

Kit Contents :

- Reagent A
- Reagent B
- Standard Protein (BSA)

Note:

- This kit contains sufficient reagents for 250 tests with 2ml reaction volume.
- Should either Reagent A or Reagent B precipitate upon shipping in cold weather or during long-term storage, dissolve the precipitate by gently warming the solution with stirring.
- If any of the kit components shows discoloration or evidence of microbial contamination, discard the reagent.
- This kit is not suitable for estimation of protein concentration less than 2 µg.
- The following substances have been reported to interfere with the accurate estimation of protein concentration with the BCA Protein Estimation Reagent. These should be avoided as components of the sample buffer: Ascorbic Acid, Catecholamines, Creatinine, Cysteine, EGTA, Impure Glycerol, Hydrogen peroxide, Hydrazides (Na_2BH_4 and $NaCNBH_3$), Iron, Lipids, Melibiose, Phenol Red, Impure Sucrose, Tryptophan, Tyrosine, Uric Acid.

Application :

- For Total Protein estimation and determining the unknow concentration using a standard BSA.

References :

- Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C (1985). Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76 85.
- Wiechelman, K., Braun, R. and Fitzpatrick, J. (1988). Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. Anal Biochem. 175, 231-237.
- Brown, R., Jarvis, K. and Hyland, K. (1989). Protein measurement using bicinchoninic acid: elimination of interfering substances. Anal. Biochem.
- Peterson, G.L. (1979) . Review of the folin phenol protein quantitation method of Lowry, Rosebrough, Farr and Randall. Anal. Biochem. 100, 201-220.

Kirschbaum, G. (1986). Use of the bicinchoninic acid assay in measuring urinary proteins. Clin. Chem. 32, No. 3, Letter to the Editor, 572.

Ordering Information:

Cat. No	PI No.	Product Description
2603100011730	KT31	Protein Estimation Kit by BCA Method, 250 reactions

Protein Estimation Kit by Bradford Macro Method

Description :

Bradford protein assay is a simple and accurate spectophotometric/colorimetric procedure for determining the concentration of protein in solution using the Coomassie G-250 dye. The Bradford method of estimation of proteins was first described by Dr Marion Bradford in 1976. The method is based on the principle of binding of Coomassie G-250 dye to proteins. The dye exists in three forms: cationic (red), neutral (green), and anionic (blue). Under acidic conditions, the dye is predominantly in the doubly protonated red cationic form (Amax = 465 nm). When the dye binds to a protein in an acidic environment, it is converted to a stable unprotonated blue form (Amax = 595 nm), resulting in a spectral shift from absorbance maximum 465 nm of reddish brown form of the dye to an absorbance maximum of 595nm which is the blue form of the dye.

The presence of certain basic amino acids such as Arginine, Lysine and Histidine in the protein are responsible for the development of color in Coomassie dye-based (Bradford) protein assays. The dye first donates its free protons to the protein being tested, causing disruption of its native state and exposing its hydrophobic sites. These hydrophobic pockets on the protein chain then bind non-covalently to the non- polar region of dye by Vander Waal forces. As a result of the proximity between positive amine group positions with the negative charge of the dye, this ionic interaction gets further strengthened and stabilizes the blue color. The amount of color complex present in the solution is an estimation of the protein concentration, which can be measured at 595nm spectrophotometrically.

The number of positive charges on the protein decides the number of dye molecules bound to the protein. Free amino acids, peptides and low molecular weight proteins do not produce color with Coomassie dye reagents. It should be noted that some chemical-protein and chemical- dye interactions interfere with the assay. Interference from non protein compounds is due to their ability to shift the equilibrium levels of the dye among the three colored species. In general, the mass of a peptide or protein to be assayed with this reagent must be at least 3000 Daltons. This protein estimation assay requires the use of a protein standard. The ideal protein to use as a standard is a purified preparation of the protein being assayed. In the absence of an absolute reference protein, another protein must be selected as a relative standard. The two most common protein standards used for protein assays are Bovine Serum Albumin (BSA) and gamma-globulin.

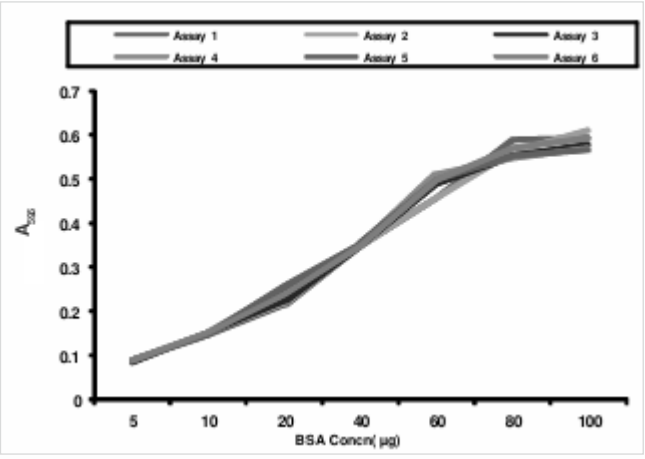
Protein Estimation Kit (By Bradford's Method) provides Bovine Serum Albumin (BSA) as the Protein Standard. The concentration of stock BSA is known and hence can be used to plot a Standard Curve of Absorbance vs concentration. This Standard Curve can be used to determine the concentration of an unknown protein between 10 µg and 150 µg using Bradford's Reagent

Note:

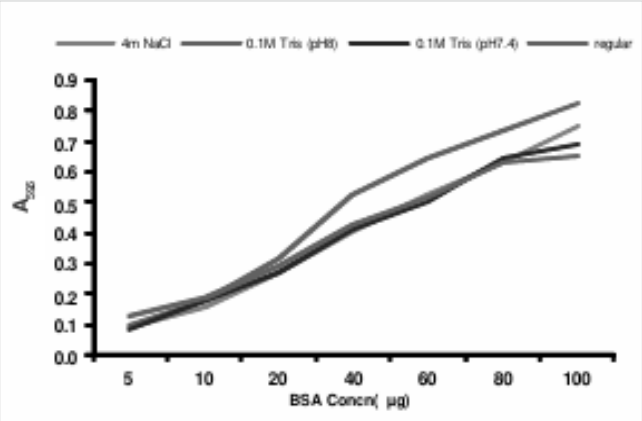
- This kit is not suitable for estimating less than 10 µg of protein.
- Presence of following in a sample are known to interfere in the assay hence should be avoided High molarity buffers of high/ low pH
- b. Detergent c. Ammonium sulphate d. Traces of Sucrose/EDTA.

Kit Contents :

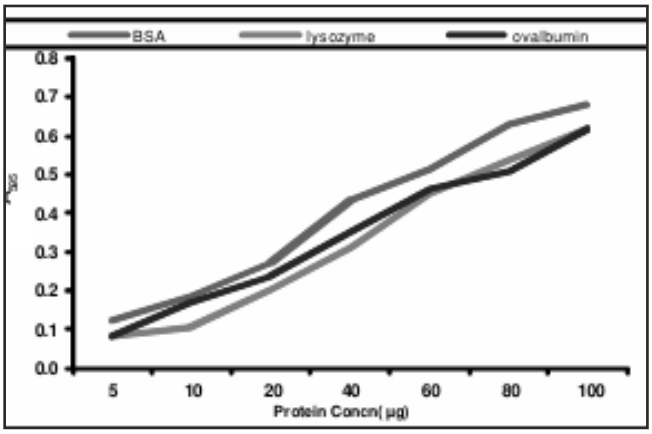
- Bradford Reagent
- Standard Protein (BSA)



A typical curve for Bovine Serum Albumin as Standard with Bradford's reagents (Performed on 6 different occasions)



A typical curve for Bovine Serum Albumin as Standard with Bradford's reagent using different buffer conditions



A typical curve for different proteins (Bovine Serum Albumin, Lysozyme and Ovalbumin) as Standard with Bradford's reagent.

References :

- Bradford, M. (1976). Anal. Biochem. 72, 248-254. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-54.
- Sedmak, J.J. and Grossberg, S.E. (1977). A rapid, sensitive and versatile assay for protein using Coomassie brilliant blue G-250. Anal. Biochem. 79:544-52.
- VanKley, H. and Hale, S.M. (1977). Anal. Biochem. 81, 485-487.
- Spector T, Refinement of the Coomassie blue method of protein quantitation. A simple and linear spectrophotometric assay for less than or equal to 0.5 to 50 micrograms of protein, Anal Biochem 86, 142-146 (1978)
- Compton, S.J. and Jones, C.J. (1985). Mechanism of dye response and interference in the Bradford protein assay. Anal. Biochem. 151:369-74.
- Messenger, M.M., et.al. (2002). J. Biol. Chem 277, 23054 23064
- Stoscheck, CM. Quantitation of Protein. Methods in Enzymology 182: 50-69 (1990).

Ordering Information:

Cat. No	PI No.	Product Description
2603300011730	KT33	Protein Estimation Kit by Bradford Macro Method, 250 reactions

Protein Estimation Kit by Lowry's Method

Description :

The Lowry protein assay is a biochemical assay for determining the total level of protein in a solution. The total protein concentration is exhibited by a color change of the sample solution in proportion to protein concentration, which can then be measured using colorimetric techniques. It is named after the biochemist Oliver H. Lowry who developed the reagent in the 1940s. The method combines the reactions of copper ions with the peptide bonds under alkaline conditions (the Biuret test) with the oxidation of aromatic protein residues. The Lowry method is best used with protein concentrations of 0.01-1.0 mg/ml. and is based on the reaction of Cu+, produced by the oxidation of peptide bonds, with Folin-Ciocalteu reagent (a mixture of phosphotungstic acid and phosphomolybdic acid in the Folin Ciocalteu reaction).

The reaction mechanism is not well understood but involves reduction of the Folin reagent and oxidation of aromatic residues (mainly tryptophan, also tyrosine). Experiments have shown that cysteine is also reactive towards the reagent. Therefore, cysteine residues in a protein probably also contribute to the absorbance seen in the Lowry Assay. The concentration of the reduced Folin reagent is measured by absorbance at 660 nm. As a result, the total concentration of protein in the sample can be deduced from the concentration of Tryptophan and Tyrosine residues that reduce the Folin reagent.

The phenolic group of Tyrosine and Tryptophan residues (amino acid) in a protein will produce a blue purple color complex. Thus the intensity of

color depends on the amount of these aromatic amino acids present and will thus vary for different proteins.

Most proteins estimation techniques use Bovine Serum Albumin (BSA) universally as a standard protein, because of its low cost, high purity and ready availability. The Lowry's method is sensitive down to about 10 µg/ml and is probably the most widely used protein assay despite its being only a relative method , subject to interference from Tris buffer, EDTA, non-ionic and cationic detergents, carbohydrate, lipids and some salts. The incubation time is very critical for a reproducible assay. The reaction is also dependent on pH and a working range of pH 9.0 to 10.5 is essential.

The Protein Estimation Kit comes with Solution I (which contains copper solution), Solution II (which contains a alkaline tartarate solution), Solution III (which contains dilute folin reagent) and BSA.

Features :

- Best used with protein concentrations of 0.01-1.0 mg/ml.
- Sensitive down to about 10 µg/ml
- Simple assay
- No special equipment required
- Rapid and sensitive method

Application :

The Lowry Protein Estimation Kit is ideal for Lowry method users who would like the increased convenience of a stable, pre-formulated product.

Kit Contents :

- Protein Standard BSA
- Solution I
- Solution II
- Solution III
- Instruction Manual

Note:

- Presence of the following compounds in sample are known to interfere with the assay and they should be avoided.
- Substances containing amine group.
- High molarity buffers of low pH.
- The pH of reaction mixture should be in the range of 10-10.5.
- Detergents. -Ammonium sulphate (>3%), Sodium phosphate (>0.2M) and Cesium bicarbonate

References :

- Lowry, O.H., et al. (1951). Protein measurement with the Folin Phenol Reagent. J Biol Chem 193:267-75.
- Vallejo, C.G. and Lagunas, R. (1970). Interferences by sulfhydryl, disulfide reagents, and potassium ions on protein determination by Lowry's method. Anal Biochem 36:207-12.

Ordering Information:

Cat. No	PI No.	Product Description
2601800011730	KT18	Protein Estimation Kit by Lowry's Method, 250 reactions

GeNei™ Protein Estimation Kit
(Bradford Micro method)

Description :

Bradford's assay is a rapid and accurate method for estimation of protein concentration. Moreover, when compared with the Lowry's Method it is subject to less interference by common reagents and non-protein components of biological sample. Consequently it is useful when the amount of the unknown protein is limited.

The assay relies on the binding of the dye Coomassie blue G250 to protein. The quantity of protein can be estimated by determining the amount of dye in the blue ionic form. This is usually achieved by measuring the absorbance of the solution at 595 nm or 625 nm.

Protein Estimation Kit by Biuret method

Description :

Quantification of proteins is an integral part of proteomics and analysis of proteins. Several methods are available for protein quantification. Some are dye binding assays; others are based on alkaline copper. The Biuret method is the simplest method of protein estimation. Its sensitivity is moderately constant from protein to protein and because of its simple procedure and quick results, it can be widely used to estimate protein in a crude extract over a large range of concentration. This method can also be used to monitor the concentration of a protein during its purification.

Under alkaline conditions, substances containing two or more peptide bonds from a purple complex with copper salts in the reagent, which absorbs at A560 nm. The intensity of the charge transfer absorption bond resulting from the Cu-protein complex is linearly proportional to the mass of the protein present in the solution. The chromophore on light absorbing centre seems to be a complex between the peptide backbone and cupric ions. The Biuret reagent is made up of KOH and hydrated copper sulfate, together with potassium sodium tartrate. The test gets its name from its positive reaction with the peptide-like bonds in the Biuret molecule. The intensity of the color developed is directly proportional to the number of peptide bonds as per the Beer-Lambert Law.

Features :

- Sensitivity of assay: 0.25mg
- Range of assay:0.25-4.0 mg
- User friendly protocol to estimate given protein.
- Ready to use reagents for rapid estimation of protein concentration.
- Consistent results.
- Quantitative test

Protein Estimation Kit (Bradford's Micro Method) provides Bovine Serum Albumin (BSA) as the Protein Standard. The concentration of stock BSA is known and hence can be used to plot a Standard Curve of Absorbance vs concentration. This Standard Curve can be used to determine the concentration of an unknown protein between 1 µg and 10µg using Bradford's Reagent.

Kit Contents :

- Protein standard, BSA.
- Bradford's reagent

Note:

Presence of the following compounds in sample are known to interfere in the assay and they should be avoided.

- High molarity buffers of low/high pH or strong bases.
- Detergents.
- Ammonium sulphate > 3%.
- Sucrose > 0.2M
- EDTA > 10 mM

References :

- Bradford, M. (1976). Anal. Biochem. 72, 248-254. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-54.
- Sedmak, J.J. and Grossberg, S.E. (1977). A rapid, sensitive and versatile assay for protein using Coomassie brilliant blue G-250. Anal. Biochem. 79:544-52.
- VanKley, H. and Hale, S.M. (1977). Anal. Biochem. 81, 485-487.
- Spector T, Refinement of the Coomassie blue method of protein quantitation. A simple and linear spectrophotometric assay for less than or equal to 0.5 to 50 micrograms of protein, Anal Biochem 86, 142-146 (1978)
- Compton, S.J. and Jones, C.J. (1985). Mechanism of dye response and interference in the Bradford protein assay. Anal. Biochem. 151:369-74.
- Messenger, M.M., et.al. (2002). J. Biol. Chem 277, 23054 23064
- Stoscheck, CM. Quantitation of Protein. Methods in Enzymology 182: 50-69 (1990).

Ordering Information:

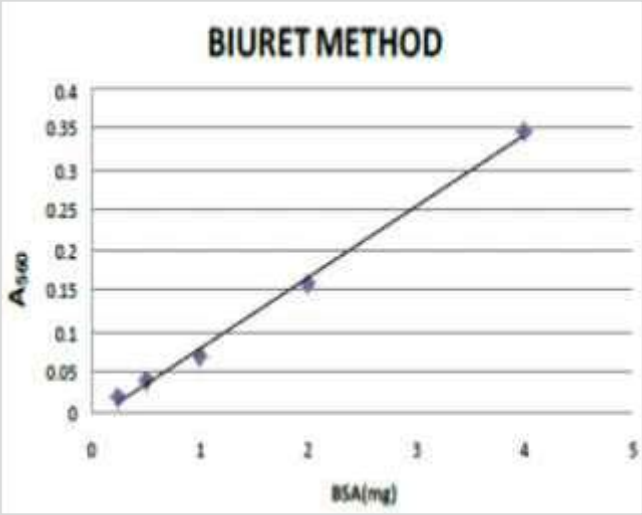
Cat. No	PI No.	Product Description
2624800021730	KT248B	GeNei™ Protein Estimation Kit (Bradford Micro method), 250 reactions

Kit Contents :

- BSA, Protein Standard
- Biuret Reagent (4X)
- Biuret Reagent Diluent

Note:

- **Sample preparation:** solubilize protein in an aqueous buffer and remove any cellular debris/ particulate matter. Take precautions to prevent microbial growth in the sample.
- Presence of the following in a sample are known to interfere with the assay and should be avoided.
  - ➢ Substances containing amine groups.
  - ➢ High molarity buffers of low pH or strong acids.
  - ➢ Detergents.
  - ➢ Ammonium sulphate > 3%.
  - ➢ Sodium phosphate > 0.2M
  - ➢ Cesium bicarbonate
- The pH of reaction mixture should be in the range of 10 - 10.5.



References :

- Gornall, A.G., Bardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 751-766
- Ryan, M.T. and Chopra, R.K. (1976) Biochim. Biophys. Acta 427, 337-349
- Ohnishi, S. T., Barr, J. K., A simplified method of quantitating protein using the biuret and phenol reagents. Anal. Biochem. 86, 193-200, (1978).
- Layne, E.Spectrophotometric and Turbidometric methods for measuring proteins. Methods in Enzymology 10:447-455, (1957).

Ordering Information:

Cat. No	PI No.	Product Description
2601900011730	KT19	Protein Estimation Kit by Biuret method, 250 reactions

Native PAGE Reagent Kit, with Marker

Description :

Proteins are commonly separated using polyacrylamide gel electrophoresis (PAGE) to characterize individual protein in a complex sample or to examine multiple proteins within a single sample. PAGE can be used as a preparative tool to obtain a pure protein sample, or as an analytical tool to provide information on the mass, charge, purity or presence of a protein. Non-denaturing PAGE, also called Native PAGE, separates proteins according to their mass-charge ratio. As there are no denaturants in native PAGE, subunit interactions within a multimeric protein are generally retained and information may be gained about the quaternary structure of the protein. Proteins may be recovered from a native gel by electro-elution.

This kit should be used for the electrophoresis of acidic and neutral proteins only. A set of 5 proteins are supplied to characterize the proteins separated in poly-acrylamide gel in their native state. The proteins supplied have a range of size from 18.4kD to 240kD. Ovalbumin is characterized with two closely spaced bands (Charge isomers). The marker is ready to load marker set. 10µl of each protein should be loaded in each well of the native gel.

Kit Contents :

- Protein Standard Marker (5 proteins)
- 30% Acrylamide
- 1.5M Tris, pH 8.8
- 0.5M Tris, pH 6.8
- Electrophoresis Buffer(10X)
- Sample Loading Buffer (5X)
- Ammonium Per-Sulphate
- TEMED

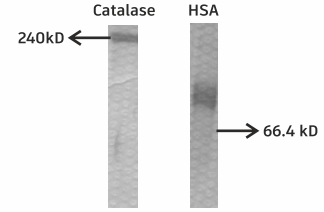
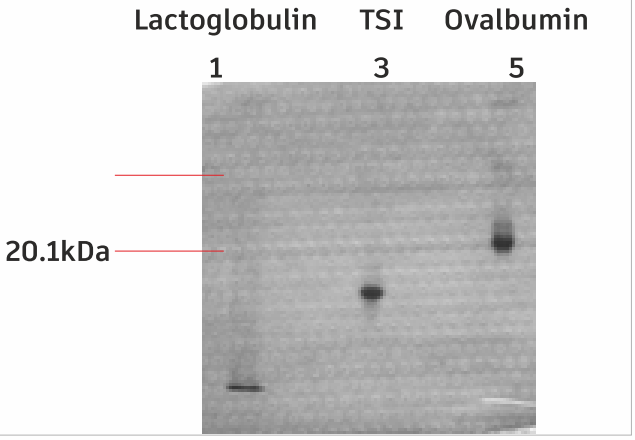


Fig 2: 8% Native PAGE indicating the separation of Catalase and HSA.

Protein	MW given in (kD)	% of gel
Catalase	240	5-8
Human Serum Albumin	66.4	5-8
Ovalbumin	43	8-10
Trypsin Soyabean Inhibitor (TSI)	20.1	8-10



10% Native PAGE indicating the separation of catalase and BSA

Reference :

- Kita, Y. and Arakawa, T. (2002). Salts and glycine increase reversibility and decrease aggregation during thermal unfolding of ribonuclease-A. Biosci. Biotechnol. Biochem. 66, 880-882.
- Eubel, H., Braun, H. P., and Millar, A. H. (2005) Blue Native PAGE in Plants: A Tool in Analysis of Protein- Protein Interactions. Plant Methods 1, 11
- Graham, J. M., and Rickwood, D. (1997) Subcellular Fractionation: A Practical Approach., OxFord University Press, Inc., New York
- Manchenko, G. P. (1994) Handbook of Detection of Enzymes on Electrophoretic Gels, CRC Press, Boca Raton
- Schagger, H. (2001) Blue Native Gels to Isolate Protein Complexes from Mitochondria. Meth. Cell Biol. 65, 231- 244.
- Schagger, H., Cramer, W. A., and von Jagow, G. (1994) Analysis of Molecular Masses and Oligomeric States of Protein Complexes by Blue Native Electrophoresis and Isolation of Membrane Protein Complexes by Two- dimensional Native Electrophoresis. Anal. Biochem. 217, 220-230.
- Schagger, H., and von Jagow, G. (1991) Blue Native Electrophoresis for Isolation of Membrane Protein Complexes in Enzymatically Active Form. Anal. Biochem. 199, 223-231.
- Zerbetto, E., Vergani, L., and Dabbeni-Sala, F. (1997) Quantification of Muscle Mitochondrial Oxidative Phosphorylation Enzymes via Histochemical Staining of Blue Native Polyacrylamide Gels.Electrophoresis 18, 2059 2064.

Ordering Information:

Cat. No	PI No.	Product Description
3163100011730	ER31	Native PAGE Reagent Kit, with Marker, for 10 PAGEs

Silver Staining Kit with Marker (Protein)

Description :

Among the various protein detection methods following electrophoresis of polyacrylamide gels, silver staining has gained wide popularity because of its sensitivity (in the very low ng range). It can be achieved with simple and cheap laboratory reagents and does not require complicated and expensive hardware for the read out. The rationale of silver staining is quite simple. Protein bound to silver ions which can be reduced under appropriate conditions to build up a visible image made of finely divided silver metal. However, silver staining is quite tricky and has its own artefacts.

The sequential phases of silver staining are :

- Protein fixation, to get rid of interfering compounds.
- Sensitization and rinses to increase sensitivity and contrast of staining.
- Silver impregnation with either silver nitrate solution or a silver ammonia complex solution.
- Rinses and development to build up the silver metal image
- Strip and rinse to end development prior to excessive background formation and to remove excess silver ion and other chemicals prior to further processing.

The kit is supplied with a Protein Marker of Medium range suitable for silver staining. The Protein Marker is supplied as a mixture of proteins as shown in figure. The concentration of each of the proteins in this mixture is 0.1 mg/ml.

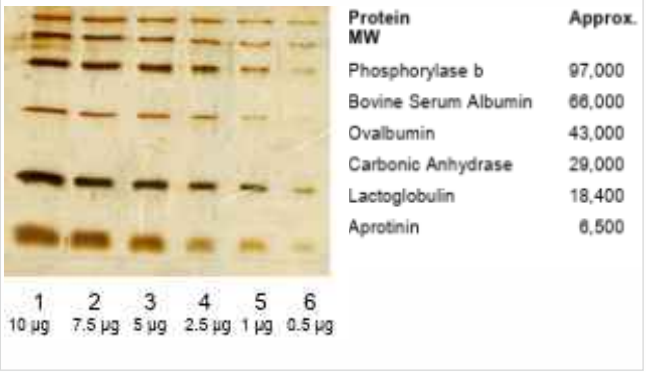
Kit Contents :

- Fixing Solution
- Wash Solution
- 500X Sensitizing Solution
- 100X Silver Staining Solution
- 2X Developing Solution
- 37% Formaldehyde

- Stop Solution
- Protein Molecular Weight Marker (Silver Staining)

Features :

- Highly sensitive.
- Detects up to nanogram level of protein.
- No background



Detection of proteins by Silver Staining: Protein Molecular Weight Marker electrophoresed on a 12% polyacrylamide.

Application :

- Silver Staining

References :

- Eschenbruch M, Burk RR. Experimentally improved reliability of ultra sensitive silver staining of protein in polyacrylamide gel. Anal Biochem 1982 Sep 1; 125 (1) 96 99.
- Merril CR, Gold man D, Sedman SA. Ebert MH. Ultra sensitive stain for protein in polyacrylamide gels shows regionalvariation in CSF proteins. Science 1981 Mar 27; 211 (4489): 1437-8.
- Chevallet M, Luche S, Rabilloud T. Silver staining of protein in polyacrylamide gels. Nat. Protoc. 2006; 1 (4): 1852-8.
- Rabilloud T. Mechanism of protein silver staining in polyacrylamide gels: a 10-year synthesis. Electrophoresis 1990 Oct; 11 (10): 785-94.

Ordering Information:

Cat. No	PI No.	Product Description
3162300011730	ER23	Silver Staining Kit with Marker (Protein), for 10 PAGEs

Western Blot Development Kit for Human Antibody

Description :

Immunoassay using enzyme labeled antibodies together with a substrate is highly specific and provides an excellent method for the detection and characterization of a particular protein in a mixture. The Western blot technique, introduced by Towbin et al in 1979, involves detection of a protein bound to a nitrocellulose or PVDF membrane using antibodies and is one of the most widely used analytical technique. A mixture of proteins is first separated by gel electrophoresis (SDS-PAGE); these proteins are then transferred to membrane. After blocking, the antigen of interest is detected by using either a labeled antibody or an unlabeled antibody followed by a labeled secondary antibody specific to the unlabelled antibody. Finally a detection method (substrate) is used to identify the antibody bound to the protein on the immobilized membrane. Biotin-Streptavidin help amplify the reaction leading to better sensitivity.

Western Blot Development Kit (For Human antibodies) utilizes direct detection technique to detect Human antibody. The antigen-immobilized membrane is incubated with its specific antibody which is of Human origin. The antibody is allowed to bind to a biotinylated secondary antibody (anti Human IgG biotin conjugate).Biotinylated secondary antibody increases the sensitivity of the reaction . Biotin has strong affinity for Streptavidin, which is labeled with Alkaline Phosphatase (ALP). The membrane is then incubated with Streptavidin ALP conjugate that binds to biotin on the antibody. This in turn is detected by BCIP/NBT, substrate specific for alkaline phosphatase. The development of blue-grey color indicates a positive reaction and hence the presence of antigen on the membrane and the antibody which has bound to it.

Features :

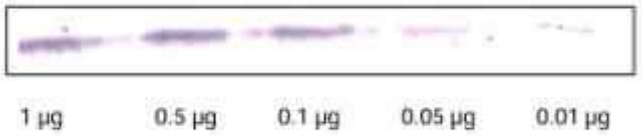
- Highly sensitive and specific due to Biotin–Streptavidin system
- Saves time, efforts
- Low background
- Control Strips enable to validate the procedure

Kit contents :

- Goat Anti-Human IgG Biotin Conjugate
- Wash Buffer (25X)
- Blocking Buffer (5X)
- Assay Buffer (10X)
- Streptavidin ALP conjugate
- BCIP/NBT substrate
- Control Strips

Applications :

- Characterization and quantitation of IgG of Human origin.
- Used to identify immobilized Human Immunoglobulin G Sensitivity of Western blot development kit for Human IgG



References :

- Towbin, H., et al. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76, 4350-4354.
- Bers, G. and Garfin, D. (1985). Protein and nucleic acid blotting and immunobiochemical detection. BioTechniques 3, 276-288.
- Bjerrum, O.J. and Heegaard, N.H.H. (1988). Handbook of Immunoblotting of Proteins. Volume 1. Technical Descriptions. CRC Press, Boca Raton.
- Gershoni, J. (1988). Protein blotting. Meth. Biochem. Anal. 33, 1-58.
- Gershoni, J.M. and Palade, G.E. (1983). Protein blotting: principles and applications. Anal. Biochem. 131, 1-15.
- Malik, V.S. and Lillehoj, E.P. (1994). Antibody Techniques. Academic Press, Inc., San Diego, CA.
- Ramlau, J. (1987). Use of secondary antibodies for visualization of bound primary reagents in blotting procedures. Electrophoresis 8, 398-402.
- Spinola, S.M. and Cannon, J.G. (1985). Different blocking agents cause variation in the immunologic detection of proteins transferred to nitrocellulose membranes. J. Immunol. Meth. 81, 161.
- Towbin, H. and J. Gordon. 1984. J. Immunol. Meth. 72:313-340

Ordering Information:

Cat. No	PI No.	Product Description
2601300011730	KT13	Western Blot Development Kit for Human Antibody, 10 blots

Western Blot Development Kit for Mouse Antibody

Description :

Immunoassay using enzyme labelled antibodies together with a substrate is highly specific and provides an excellent method for the detection and characterization of a particular protein in a mixture. The Western blot technique, introduced by Towbin et al in 1979, involves detection of a protein bound to a nitrocellulose or PVDF membrane using antibodies and is one of the most widely used analytical technique. A mixture of proteins is first separated by gel electrophoresis (SDS-PAGE); these proteins are then transferred to membrane. After blocking, the antigen of interest is detected by using either a labelled antibody or an unlabelled antibody followed by a labelled secondary antibody specific to the unlabelled antibody. Finally a detection method (substrate) is used to identify the antibody bound to the protein on the immobilized membrane. Biotin-Streptavidin help amplify the reaction leading to better sensitivity.

Western Blot Development Kit (For mouse antibodies) utilizes direct detection technique to detect Mouse antibody. The antigen-immobilized membrane is incubated with its specific antibody which is of Mouse origin. The antibody is allowed to bind to a biotinylated secondary antibody (anti mouse IgG biotin conjugate).Biotinylated secondary antibody increases the sensitivity of the reaction . Biotin has strong affinity for Streptavidin, which is labelled with Alkaline Phosphatase (ALP). The membrane is then incubated with Streptavidin

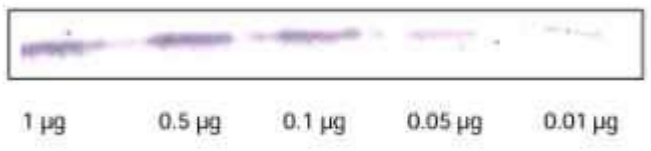
ALP conjugate that binds to biotin on the antibody. This in turn is detected by BCIP/NBT, substrate specific for alkaline phosphatase. The development of blue-grey color indicates a positive reaction and hence the presence of antigen on the membrane and the antibody which has bound to it.

Features :

- Highly sensitive and specific due to Biotin–Streptavidin system
- Saves time, efforts
- Low background
- Control Strips enable to validate the procedure

Kit Contents :

- Goat Anti-Mouse IgG Biotin Conjugate
- Wash Buffer (25X)
- Blocking Buffer (5X)
- Assay Buffer (10X)
- Streptavidin ALP conjugate
- BCIP/NBT substrate
- Control Strips



Sensitivity of Western blot development kit for Mouse IgG

References :

- Towbin, H., et al. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76, 4350-4354.
- Bers, G. and Garfin, D. (1985). Protein and nucleic acid blotting and immunobiochemical detection. BioTechniques 3, 276-288.
- Bjerrum, O.J. and Heegaard, N.H.H. (1988). Handbook of Immunoblotting of Proteins. Volume 1. Technical Descriptions. CRC Press, Boca Raton.
- Gershoni, J. (1988). Protein blotting. Meth. Biochem. Anal. 33, 1-58.
- Gershoni, J.M. and Palade, G.E. (1983). Protein blotting: principles and applications. Anal. Biochem. 131, 1-15.
- Malik, V.S. and Lillehoj, E.P. (1994). Antibody Techniques. Academic Press, Inc., San Diego, CA.
- Ramlau, J. (1987). Use of secondary antibodies for visualization of bound primary reagents in blotting procedures. Electrophoresis 8, 398-402.
- Spinola, S.M. and Cannon, J.G. (1985). Different blocking agents cause variation in the immunologic detection of proteins transferred to nitrocellulose membranes. J. Immunol. Meth. 81, 161.
- Towbin, H. and J. Gordon. 1984. J. Immunol. Meth. 72:313-340

Ordering Information:

Cat. No	PI No.	Product Description
2601400011730	KT14	Western Blot Development Kit for Mouse Antibody, 10 blots

Protein Electrophoresis & Western Blotting Reagents.

Pre-stained Protein Marker

Introduction :

Pre-stained Protein Marker is a mixture of purified and pre-stained proteins whose molecular weights are well calibrated. It is available in a ready to use format (boiling not required) and is suitable for sizing proteins in SDS PAGE.

A coloured chromophore is covalently bound to all the proteins, which are visible during electrophoresis or electrophoretic transfer from the gel to a membrane as blue bands. A green, pink, and orange reference band, respectively, enable easier identification.

Features :

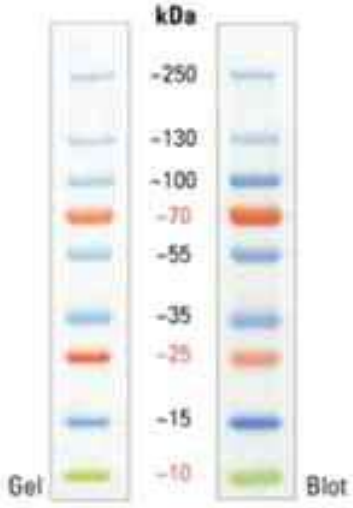
- Monitoring protein transfer onto membranes after Western blotting.
- Monitoring protein migration during SDS PAGE.
- Sizing of proteins on SDS PAGE and Western blots.

Storage: -20°C.

Genei has different range of Protein marker to meet customer requirement.

1. Pre-Stained Protein Marker, 10-250kD

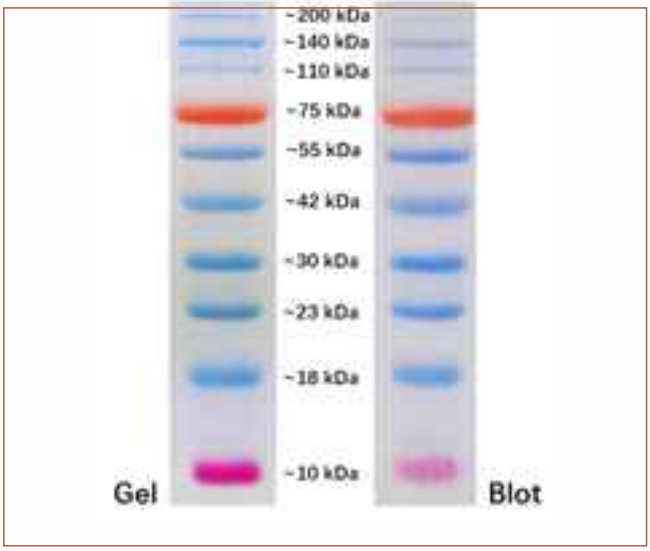
Specifications	
Number of bands	9
Product Type	Protein ladder
Ready to Use	Yes
Size Range	10-250kD
Gel Compatibility	Tris-Glycine Gels, SDS PAGE Gels
Stain Type	6 blue, 2 orange and 1 green
Molecular Weight	250, 130, 100, 75, 55, 35, 25, 15, 10kD
Quantity	250µl
System Type	Western Blotting, SDS-PAGE



12% Tris Glycine SDS PAGE

2. Pre-Stained Protein Marker, 10-200kD

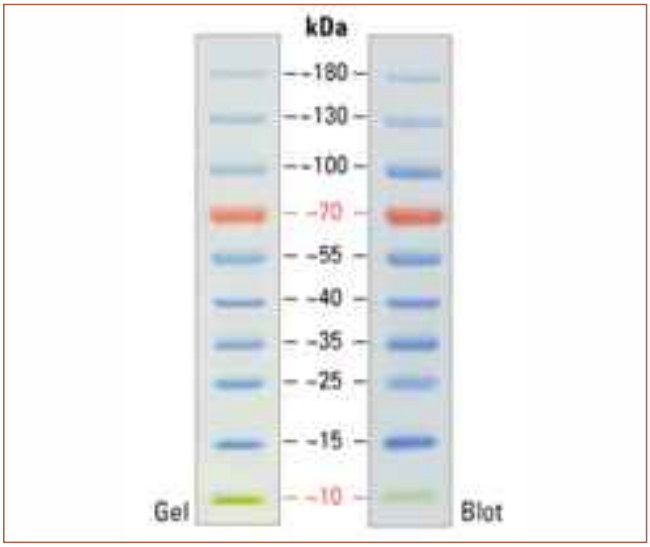
Specifications	
Number of bands	10
Product Type	Protein ladder
Ready to Use	Yes
Size Range	10-200kD
Gel Compatibility	Tris-Glycine Gels, SDS PAGE Gels
Stain Type	8 blue, 1 orange and 1 pink
Molecular Weight	200, 140, 110, 75, 55, 42, 30, 23, 18, 10kD
Quantity	250µl
System Type	Western Blotting, SDS-PAGE



12% Tris Glycine SDS PAGE

3. Pre-Stained Protein Marker, 10-180kD

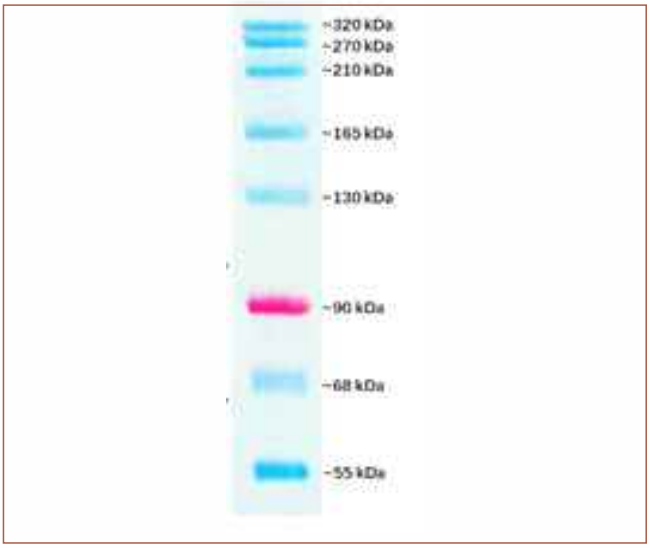
Specifications	
Number of bands	10
Product Type	Protein ladder
Ready to Use	Yes
Size Range	10-180kD
Gel Compatibility	Tris-Glycine Gels, SDS PAGE Gels
Stain Type	8 blue, 1 orange and 1 green
Molecular Weight	180, 130, 100, 70, 55, 40, 35, 25, 15, 10kD
Quantity	250µl
System Type	Western Blotting, SDS-PAGE



10% Tris Glycine SDS PAGE

4. Pre-Stained Protein Marker, 55-320kD

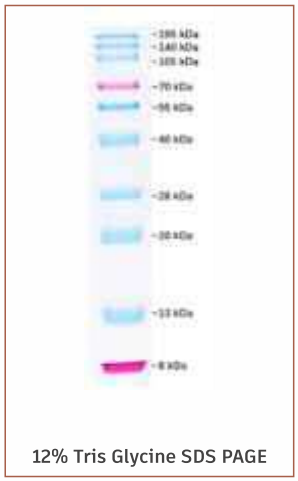
Specifications	
Number of bands	8
Product Type	Protein ladder
Ready to Use	Yes
Size Range	55-250kD
Gel Compatibility	Tris-Glycine Gels, SDS PAGE Gels
Stain Type	7 blue, 1 pink
Molecular Weight	320, 270, 210, 165, 130, 90, 68, 55kD
Quantity	250µl
System Type	Western Blotting, SDS-PAGE



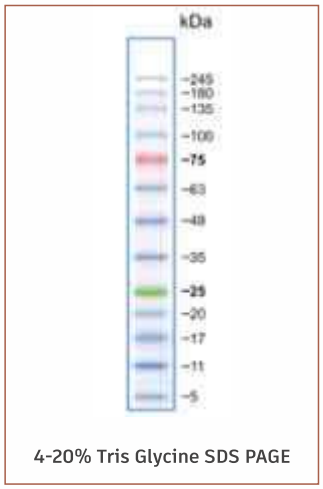
6% Tris Glycine SDS PAGE

5. Pre-Stained Protein Marker, 8-195kD

Specifications	
Number of bands	10
Product Type	Protein ladder
Ready to Use	Yes
Size Range	8-195kD
Gel Compatibility	Tris-Glycine Gels, SDS PAGE Gels
Stain Type	8 blue, 2 pink
Molecular Weight	195, 140, 105, 70, 55, 40, 28, 20, 13, 8kD
Quantity	250µl
System Type	Western Blotting, SDS-PAGE



12% Tris Glycine SDS PAGE



4-20% Tris Glycine SDS PAGE

6. Pre-Stained Protein Marker, 3.5 - 245kD

Specifications	
Number of bands	13
Product Type	Protein ladder
Ready to Use	Yes
Size Range	3.5 - 245kD
Gel Compatibility	Tris-Glycine Gels, SDS PAGE Gels
Stain Type	11 blue, 1 red and 1 green
Molecular Weight	245, 180, 135, 100, 75, 63, 48, 35, 25, 20, 17, 11, 3.5kD
Quantity	250µl
System Type	Western Blotting, SDS-PAGE

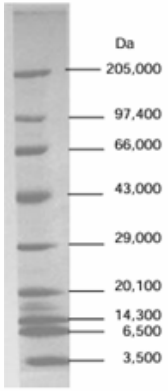
Ordering Information:

Product	Cat #	PI No.
Pre-stained Protein Marker, 0.25ml; 10-180kD	3110482501730	PPMWA
Pre-stained Protein Marker, 0.25ml; 10-200kD	3110492501730	PPMWB
Pre-stained Protein Marker, 0.25ml; 10-250kD	3110502501730	PPMWC
Pre-stained Protein Marker, 0.25ml; 55-320kD	3110512501730	PPMWD
Pre-stained Protein Marker, 0.25ml; 8-195kD	3110522501730	PPMWE
Pre-stained Protein Marker, 0.25ml; 3.5-245kD	3110532501730	PPMWF

Protein Molecular Weight Marker, Broad Range

Description :

Protein molecular weight marker is a mixture of individually purified proteins mixed to give bands of approximately equal intensity when electrophoresed on a sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE). The marker is supplied in 10 mM Phosphate buffer (pH 7.0), 0.15 M KCl and 50% glycerol. The protein, their respective molecular weights (MW) are as follows



Sl.No.	Protein	MW (Da)
1	Myosin, Rabbit Muscle	205,000
2	Phosphorylase b	97,400
3	Bovine Serum Albumin	66,000
4	Ovalbumin	43,000
5	Carbonic Anhydrase	29,000
6	Soyabean Trypsin Inhibitor	20,100
7	Lysozyme	14,300
8	Aprotinin	6,500
9	Insulin (α and β chains)	3,500

10 µl of protein molecular weight marker visualized on a 8 - 20% gradient SDS-PAGE after coomassie blue staining.

Storage: -20C

Note:

- Always store marker at -20° C.
- Mix content of the vial by tapping before pipetting and return the vial to -20° C immediately.
- Repeated freeze -thaw is not recommended.

Applications :

- The protein molecular weight marker can be used to size molecular weights of unknown proteins

References :

- Doucet, A. & Beauregard, M. A sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein ladder made of disulfide-bridged proteins. Anal. Biochem
- Harold H (1951). "Origin of the Word 'Protein.'". Nature. 168

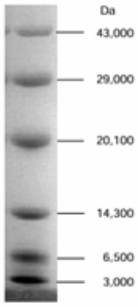
Ordering Information:

Cat. No	PI No.	Product Description
3110475001730	PMWB	Protein Molecular Weight Marker, Broad Range (50 lanes), 0.5 ml
3110472501730	PMWB1	Protein Molecular Weight Marker, Broad Range (25 lanes), 0.25 ml

Protein Molecular Weight Marker, Lower Range

Description :

Protein molecular weight marker is a mixture of individually purified proteins mixed to give bands of approximately equal intensity when electrophoresed on a sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE). The marker is supplied in 10 mM Phosphate buffer (pH 7.0), 0.06 M KCl and 50% glycerol. The protein, their respective molecular weights (MW) are as follows.



Sl.No.	Protein	MW (Da)
1	Ovalbumin	43,000
2	Carbonic Anhydrase	29,000
3	Soyabean Trypsin Inhibitor	20,100
4	Lysozyme	14,300
5	Aprotinin	6,500
6	Insulin (α and β chains)	3,500

10 µl of protein molecular weight marker visualized on a 12% SDS-PAGE after Coomassie blue staining.

Related Product:-RPMWM :

The marker is supplied in 25 mM Tris-HCl pH 6.8, 0.8% SDS, 2% -mercaptoethanol, 0.004% bromophenol blue and 50% glycerol. The marker is supplied in gel loading buffer and does not require boiling.

Storage: -20C

Note:

- Always store marker at -20° C.
- Mix content of the vial by tapping before pipetting and return the vial to -20° C immediately.
- Repeated freeze -thaw is not recommended.
- A faint band above Ovalbumin (43 kDa) may be observed.

Applications :

- The protein molecular weight marker can be used to size molecular weights of unknown proteins

References :

- Doucet, A. & Beauregard, M. A sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein ladder made of disulfide-bridged proteins. Anal. Biochem
- Harold H (1951). "Origin of the Word 'Protein.'". Nature. 168

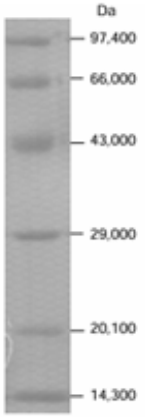
Ordering Information:

Cat. No	PI No.	Product Description
3110175001730	PMWL	Protein Molecular Weight Marker, Lower Range (50 lanes), 0.5 ml

Protein Molecular Weight Marker, Medium Range

Description :

Protein molecular weight marker is a mixture of individually purified proteins mixed to give bands of approximately equal intensity when electrophoresed on a sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE). The marker is supplied in 10 mM Phosphate buffer (pH 7.0), 0.06 M KCl and 50% glycerol. The protein, their respective molecular weights (MW) are as folllows.



Sl.No.	Protein	MW (Da)
1	Phosphorylase b	97,400
2	Bovine Serum Albumin	66,000
3	Ovalbumin	43,000
4	Carbonic Anhydrase	29,000
5	Soyabean Trypsin Inhibitor	20,100
6	Lysozyme	14,300

10 µl of protein molecular weight marker visualized on a 15% SDS-PAGE after Coomassie blue staining.

Related Product:-RPMWM :

The marker is supplied in 25 mM Tris-HCl pH 6.8, 0.8% SDS, 2% -mercaptoethanol, 0.004% bromophenol blue and 50% glycerol. The marker is supplied in gel loading buffer and does not require boiling.

Storage: -20C

Note:
- Always store marker at -20°C.
- Mix content of the vial by tapping before pipetting and return the vial to -20°C immediately.
- Repeated freeze -thaw is not recommended.

Applications :

- The protein molecular weight marker can be used to size molecular weights of unknown proteins

References :

- Doucet, A. & Beauregard, M. A sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein ladder made of disulfide-bridged proteins. Anal. Biochem
- Harold H (1951). "Origin of the Word 'Protein.'". Nature. 168

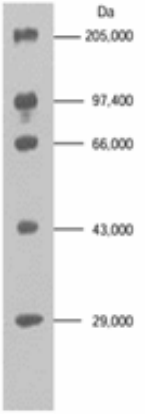
Ordering Information:

Cat. No	PI No.	Product Description
3110275001730	PMWM	Protein Molecular Weight Marker, Medium Range (50 lanes), 0.5 ml
3111275001730	RPMWM	Ready to use PMW Marker, Medium Range, 25 lanes, 0.5ml

Protein Molecular Weight Marker, Higher Range

Description :

Protein molecular weight marker is a mixture of individually purified proteins mixed to give bands of approximately equal intensity when electrophoresed on a sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE). The marker is supplied in 10 mM Phosphate buffer (pH 7.0), 0.06 M KCl and 50% glycerol. The protein, their respective molecular weights (MW) are as follows:



Sl.No.	Protein	MW (Da)
1	Myosin, Rabbit Muscle	205,000
2	Phosphorylase b	97,400
3	Bovine Serum Albumin	66,000
4	Ovalbumin	43,000
5	Carbonic Anhydrase	29,000

10 µl of protein molecular weight marker visualized on a 8% SDS-PAGE after Coomassie blue staining.

Storage: -20C

Related Product:-RPMWH :

The marker is supplied in 25 mM Tris-HCl pH 6.8, 0.8% SDS, 2% -mercaptoethanol, 0.004% bromophenol blue and 50% glycerol. The marker is supplied in gel loading buffer and does not require boiling.

Note:

- Always store marker at -20°C.
- Mix content of the vial by tapping before pipetting and return the vial to -20°C immediately.
- Repeated freeze -thaw is not recommended.

Applications :

- The protein molecular weight marker can be used to size molecular weights of unknown proteins

References :

- Doucet, A. & Beauregard, M. A sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein ladder made of disulfide-bridged proteins. Anal. Biochem
- Harold H (1951). "Origin of the Word 'Protein.'". Nature. 168

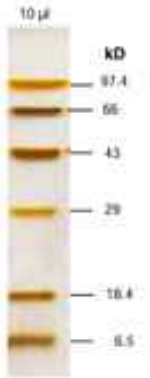
Ordering Information:

Cat. No	PI No.	Product Description
3110375001730	PMWH	Protein Molecular Weight Marker, Higher Range (50 lanes), 0.5 ml
3111375001730	RPMWH	Ready to use PMW Marker, Higher Range, 25 lanes, 0.5ml

Protein Molecular Weight Marker, for Silver Staining

Description :

Protein molecular weight marker is a mixture of individually purified proteins mixed to give bands of approximately equal intensity when electrophoresed on a sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE). The marker is supplied in 10 mM phosphate buffer (pH 7.0), 45 mM KCl and 50% glycerol. The protein, their respective molecular weights (MW) are as follows



12% SDS PAGE

Sl.No.	Protein	MW (Da)
1	Phosphorylase b	97,400
2	Bovine Serum Albumin	66,000
3	Ovalbumin	43,000
4	Carbonic Anhydrase	29,000
5	Lactoglobulin	18,400
6	Aprotinin	6,500

Storage: -20°C

Note:

- Aprotinin may appear as doublet.
- Intensity of individual band will depend on staining method. Increase load volume to 15-20 µl. If any of the bands appear faint on staining.
- Always store marker at -20°C.
- Mix content of the vial by tapping before pipetting and return the vial to -20°C immediately
- Repeated freeze -thaw is not recommended.

References :

- Switzer RC 3rd, Merril CR, Shifrin S (Sep 1979). "A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels". Anal.
- Hempelmann E, Schulze M, Götze O (1984). "Free SH-groups are important for the polychromatic staining of proteins with silver nitrate.
- Lelong C, Chevallet M, Luche S, Rabilloud T (2009). Silver staining of proteins in 2DE gels

Ordering Information:

Cat. No	PI No.	Product Description
3110572501730	PMWSS	Protein Molecular Weight Marker, for Silver Staining (25 lanes), 0.25 ml

Native PAGE Protein Molecular Weight Marker

Description :

Native PAGE Protein molecular weight marker is a set of five proteins with molecular weight ranging from 240kD to 18.4kD, to characterize the proteins separated in poly-acrylamide gels in their native state.

Protein MW (Da) Recommended % of gel

Sl.No.	Protein	MW (Da)	Gel Percentage
1	Catalase	240,000	5-8
2	Human Serum Albumin	66400	5-8
3	Ovalbumin	43,000	8-10
4	Soyabean Inhibitor	20,100	8-10
5	Lactoglobulin	18,400	8-10

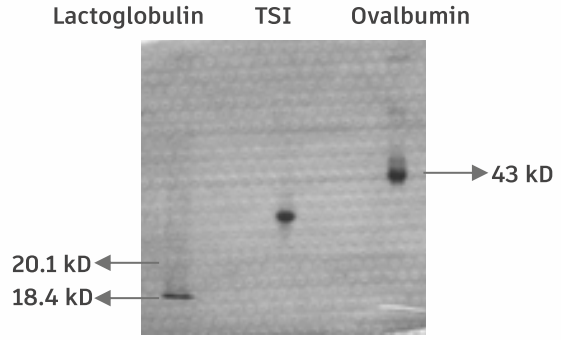


Fig1: 10% Native PAGE indicating the separation of Lactoglobulin, Ovalbumin and TSI.

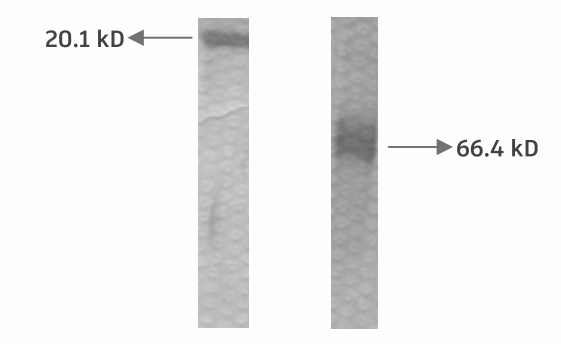


Fig 2: 8% Native PAGE indicating the separation of Catalase and HSA.

Note:
- Always store the marker at -20° C.
- Repeated freeze -thaw is not recommended.
- Mix content of the vial by tapping before pipetting and return the vial to -20° C immediately.
- Protein Marker should be electrophoresed at 50V.
- Avoid heating of apparatus during electrophoresis to prevent splitting of protein bands.
- Ovalbumin and Lactoglobulin are observed as two closely spaced bands.(charge isomers)
- Do not mix the proteins & load.

References :

- Determination of molecular weight of native proteins by polyacrylamide gradient gel electrophoresis-H Nishizawa 1, N Kita, S Okimura, E Takao, Y Abe.

Ordering Information:

Cat. No	PI No.	Product Description
3110600011730	PMWN	Native PAGE Protein Molecular Weight Marker, 5 x 0.5 ml

Acrylamide

Features :

- **Formula:** $CH_2CHCONH_2$
- **Molecular Weight :** 71.08
- **Appearance :** White crystalline powder.
- **Purity :** 99%.

Storage : Room Temperature.

Note: Neurotoxic, always handle with glove..

Ordering Information:

Cat. No	PI No.	Product Description
3171301001730	ER13	Acrylamide, 100 g

Bis-acrylamide

Description :

N,N,-Methylene-bis-acrylamide (bis-acrylamide) serves as a crosslinking agent during polymerization of acrylamide.

- **Formula :** C7H10O2N2
- **Molecular Weight :** 154.2
- **Appearance :** White powder.
- **Purity :** 98%.
- **Acrylic acid content :** < 001%
- **Conductance of 2% Solution:** < 10 µmho
- **Store :** Room Temperature.

Note: Neurotoxic, always handle with glove..

Ordering Information:

Cat. No	PI No.	Product Description
3171301001730	ER13	Acrylamide, 100 g

Acrylamide/ Bis-acrylamide solution 30%, 19:1 ratio

Description :

Acrylamide/Bisacrylamide Solution 19:1 ratio is ready to use Acrylamide Solution for preparation of Polyacrylamide Gels for protein electrophoresis. The stock solution can be used for making gel of desired percentage.

Storage: 4°C

Gel	Separating					Stacking
Volume	10 ml					5 ml
Acrylamide Conc.	6%	8%	10%	12%	15%	5%
Deionised water	5.4	4.7	4.1	3.4	2.4	3.45
30% Acrylamide	2.0	2.7	3.3	4.0	5.0	0.83
Separating gel buffer	2.5	2.5	2.5	2.5	2.5	--
Stacking gel buffer	--	--	--	--	--	0.63
20% APS	0.05	0.05	0.05	0.05	0.05	0.025
TEMED	0.008	0.006	0.004	0.004	0.004	0.005

Quality control assays :

- Performance Test: 12% SDS-PAGE was prepared with acrylamide/bisacrylamide solution and Protein Molecular Weight Marker, Medium Range (PMWM) was electrophoresed. Sharp, well separated protein bands of PMWM were observed on staining with Ezee Blue Gel Stainer.

Note: Neurotoxic , always handle with gloves

References :

• https://cshprotocols.cshlp.org/content/2006/1/pdb.rec10481

Ordering Information:

Cat. No	PI No.	Product Description
3100181001730	ER01	Acrylamide/Bis-acrylamide solution 30%, 19:1 ratio, 100 ml

Acrylamide/ Bis-acrylamide solution 30%, 29:1 ratio

Description :

Acrylamide/Bisacrylamide Solution 29:1 ratio is ready to use Acrylamide Solution for preparation of Polyacrylamide Gels for protein electrophoresis. The stock solution can be used for making gel of desired percentage.

Storage: 4°C

Gel	Separating					Stacking
Volume	10 ml					5 ml
Acrylamide Conc.	6%	8%	10%	12%	15%	5%
Deionised water	5.4	4.7	4.1	3.4	2.4	3.45
30% Acrylamide	2.0	2.7	3.3	4.0	5.0	0.83
Separating gel buffer	2.5	2.5	2.5	2.5	2.5	--
Stacking gel buffer	--	--	--	--	--	0.63
20% APS	0.05	0.05	0.05	0.05	0.05	0.025
TEMED	0.008	0.006	0.004	0.004	0.004	0.005

Quality control assays :

Performance Test: 12% SDS-PAGE was prepared with acrylamide/bisacrylamide solution and Protein Molecular Weight Marker, Medium Range (PMWM) was electrophoresed. Sharp, well separated protein bands of PMWM were observed on staining with Ezee Blue Gel Stainer.

Note: Neurotoxic , always handle with gloves

References :

•https://cshprotocols.cshlp.org/content/2006/1/pdb.rec10481

Ordering Information:

Cat. No	PI No.	Product Description
3100281001730	ER02	Acrylamide/Bis-acrylamide solution 30%, 29:1 ratio, 100 ml
3100210001730	ER02B	Acrylamide/Bis-acrylamide solution 30%, 29:1 ratio, 1000 ml

Ammonium persulphate (APS)

Description :

Ammonium persulfate is used for preparation of polyacrylamide gels as polymerization initiator.

- **Formula :** $(NH4)_2S_2O_8$
- **Molecular Weight :** 228.2
- **Appearance :** White powder.

Storage: Room Temperature (Desiccate)

Notes: Ammonium persulfate decays slowly in solution, so replace the stock solution every 2-3 weeks

- Dissolve the content of each vial (i.e 100 mg pack) in 0.5 ml deionized water.
- Incase 10 g pack, weigh 100 mg in 0.5 ml deionized water.Store the resuspended samples at 4° C, use within a week after reconstitution.

Ordering Information:

Cat. No	PI No.	Product Description
3171100101730	ER11B	Ammonium persulphate (APS) (Bulk pack), 10 g
3171100077730	ER11	Ammonium persulphate (APS) (Jumbo pack), 10 X 100g

TEMED

Description :

TEMED - N, N, N', N' tetramethylethylenediamine Light sensitive, store in dark. 5 ml Polymerization accelerator. Ammonium persulfate is used as a catalyst for the copolymerization of acrylamide and bisacrylamide gels. The polymerization reaction is driven by free radicals generated by an oxido-reduction reaction in which a diamine (e.g., TEMED) is used as the adjunct catalyst

Store: Room Temperature

Reference :

- https://cshprotocols.cshlp.org/content/2006/1/pdb.rec8237.full

Ordering Information:

Cat. No	PI No.	Product Description
3171280051730	ER12S	TEMED, 5 ml

Thimerosal

Description :

Thiomersal (also known as thimerosal, Merthiolate) is an organomercurial derivative of ethyl mercury that has been used very widely, and for a very long time, as a preservative in vaccines in their bulk formulations. Its primary purpose has been to prevent microbial growth in the product during storage and use. It has also been used during vaccine production both to inactivate certain organisms and toxins and to maintain a sterile production line.

Store: Room temperature ($_{15}$° - $_{30}$° C).

Ordering Information:

Cat. No	PI No.	Product Description
2131600011730	FC104	Thimerosal, 1kG

Tris-SDS, pH 8.8

Description :

Tris–SDS pH 8.8 is a ready to use buffer used for preparation of Polyacrylamide Gel (i.e Separating Gel Mix) used for analysis of proteins. Its pKa of 8.1 makes it an excellent buffer in the 7-9 pH range. This makes it a good choice for most biological systems. SDS in the buffer helps keep the proteins linear. In the resolving gel, the pH changes from 6.8 to 8.8 and the pores are smaller. As pH increases, the N-terminal amino groups are deprotonated. Amino acids and proteins are more negatively charged at equilibrium than in stacking gel. As a result, glycine moves faster than proteins and helps the protein to resolve faster with .

Quality control assays :

- Performance Test: 12% SDS-PAGE was prepared with acrylamide/bis-acrylamide solution and Protein Molecular Weight Marker, Medium Range (PMWM) was electrophoresed. Sharp, well separated protein bands of PMWM were observed on staining with Ezee Blue Gel Stainer.

Store: 4°C

Ordering Information:

Cat. No	PI No.	Product Description
3100481001730	ER04	Tris-SDS, pH 8.8, 100 ml, 1.5M

Tris-SDS, pH 6.8

Description :

Tris –SDS pH 6.8 is use to prepare Stacking Gel Solution of Polyacrylamide Gel Electrophoresis used for analysis of proteins. Stacking gel is a low concentrated polyacrylamide gel that is placed on the top of more concentrated resolving gel (separating gel) in SDS-PAGE technique. The stacking gel is used to improve the resolution of electrophoresis. The resolution increases because of the difference between concentrations of stacking gel and resolving gel that effect on the proteins in the sample. Since the concentration of polyacrylamide in stacking gel is low, the pore size is higher. This also helps in increasing the separation.

The stacking gel is a loose polyacrylamide gel with big pores of about 5% polyacrylamide. These pores do not act as a considerable barrier for large protein molecules. Hence this gel affects slightly on the mobility of those proteins. This makes separation according to the mobility and size of protein by concentrating them in between two gels. The pH of the stacking gel is 6.8. Its pH is acidic than that of resolving gel by 2 pH units. This pH implies a lower ionic strength hence a higher electrical resistance. This provokes the mobility of proteins than other charged particles present in the gel.

Quality control assays :

- Performance Test: 12% SDS-PAGE was prepared with Tris SDS pH 8.8 and Protein Molecular Weight Marker, Medium Range (PMWM) was electrophoresed. Sharp, well separated protein bands of PMWM were observed on staining with Ezee Blue Gel Stainer.

Store: 4°C

Ordering Information:

Cat. No	PI No.	Product Description
3100581001730	ER05	Tris-SDS, pH 6.8, 100 ml , 1M

Tris-glycine-SDS Buffer

Description :

Tris –Glycine SDS Buffer (Gel Running Buffer) is used for electrophoresis of protein sample on polyacrylamide gel. Solution is supplied as 10X Concentration.

Quality control assays :

- Performance Test: 12% SDS-PAGE was prepared with acrylamide/bisacrylamide solution and Protein Molecular Weight Marker, Medium Range (PMWM) was electrophoresed. Sharp, well separated protein bands of PMWM were observed on staining with Ezee Blue Gel Stainer

Storage: Room Temperature ◆ Do not store in the refrigerator

References :

- https://cshprotocols.cshlp.org/content/2014/7/pdb.rec081117.full

Ordering Information:

Cat. No	PI No.	Product Description
3100690011730	ER06	Tris-glycine-SDS Buffer, 1000 ml, 10X

Sample Loading Buffer for SDS PAGE

Description :

Sample Loading Buffer is required to electrophorese protein sample in denatured condition and the blue color dye also helps to track the running status of the samples at any point of time. The solution is supplied at 5X Concentration.

Quality control assays :

- Performance Test: 12% SDS-PAGE was prepared with acrylamide/bisacrylamide solution and Protein Molecular Weight Marker, Medium Range (PMWM) was electrophoresed. Sharp, well separated protein bands of PMWM were observed on staining with Ezee Blue Gel Stainer

References :

- Modification of the Laemmli Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis Procedure to Eliminate Artifacts on Reducing and Nonreducing Gels.

Store: 4°C

Ordering Information:

Cat. No	PI No.	Product Description
3100780101730	ER07	Sample Buffer for SDS PAGE (2ml x 5 Nos), 5X

10X Tris Tricine SDS Buffer

Description :

Tris-Tricine-SDS running buffer is the combination of Cathode (upper reservoir) buffer and Anode (lower reservoir) buffer for SDS-polyacrylamide gel electrophoresis of proteins using the Schagger and von Jagow method. The Schagger and von Jagow method is designed for the separation of small molecular weight proteins. It differs from the Laemmli method in that the glycine is replaced with tricine.

Quality control assays :

- Performance Test: 16.5% SDS-PAGE was prepared with acrylamide/bisacrylamide solution and Protein Molecular Weight Marker, Lower Range (PMWL) was electrophoresed. Sharp, well separated protein bands of PMWL were observed on staining with Brilliant Blue R Gel Stainer.

Storage: Room Temperature

Ordering Information:

Cat. No	PI No.	Product Description
3103782501730	ER37	10X Tris Tricine Buffer, 250 ml

Coomassie Gel Destainer

Description :

Coomassie brilliant blue R-250 based dye stains protein bands on polyacrylamide gel reversibly. An optimal destaining with Coomassie Gel Destainer, improves the sensitivity, thereby leading to better visualization of stained protein bands on the gel.

Destainer is supplied at 4X concentration.

Storage: Room Temperature

Note: Do not store in the refrigerator, as it may cause precipitation.

Procedure :

- Gel staining: Stain the polyacrylamide gel by gently shaking with Coomassie Gel Stainer on a rocker for 1-2 hours. Overnight staining can be done if required. For a mini gel typically 20-40ml of stainer is required. For better results use Coomassie Gel Stainer at 1X concentration.
- Gel destaining: Dilute the 4X Coomassie Gel Destainer to 1X with deionized water. Rinse the gel after staining, with deionized water and destain with several changes of 1X Destainer until the bands become visible against a clear background.

Quality control assays :

- Sensitivity: Protein (BSA) was detected after 2 hours of staining followed by destaining of 12% SDS Polyacrylamide Gel.

Ordering Information:

Cat. No	PI No.	Product Description
3103385001730	ER33	Coomassie Gel Destainer, 500 ml , 4X

Coomassie Gel Stainer

Description :

Coomassie Gel Stainer is a brilliant blue R-250 based protein stain for the detection of protein bands on polyacrylamide gels. It forms strong but non-covalent intensely coloured complexes with proteins, mostly based on a combination of van der Waal's forces and electrostatic interactions. Coomassie Gel Stainer is highly sensitive and can detect as little as 0.5µg protein per cm3 of the gel. Stainer is supplied at 2X concentration.

Storage: Room Temperature Do not store in the refrigerator, as it may cause precipitation.

Features: Easy detection, higher sensitivity and reversible staining.

Procedure :

- Gel rinsing: Place the gel in a suitable tray and rinse with deionized water 3 times for 5 minutes each, to remove the SDS in the gel to avoid background.
- Gel staining: Dilute 2X Coomassie Gel Stainer to 1X with deionized water. For a mini gel typically 20-40 ml of stainer is required. Gently shake the gel by placing the tray on a rocker for 1-2 hours. Overnight staining can be done if required.
- Gel destaining: For optimal results destain the gel with several changes of 1X Coomassie gel destainer until the bands become visible against a clear background.

Quality control assays :

- Sensitivity: Protein (BSA) was detected after 2 hours of staining followed by destaining of 12% SDS Polyacrylamide Gel.

Ordering Information:

Cat. No	PI No.	Product Description
3103485001730	ER34	Coomassie Gel Stainer, 500 ml , 2X

10 X PBS, pH 7.2

Description :

Phosphate buffered saline (abbreviated PBS) is a buffer commonly used as a diluent as well as Wash Buffer in ELISA applications. It is supplied at 10X concentration. Dilute to 1X before use.

Store: 4° C

Direction to Use: Dilute by adding 1 part 10X PBS to 9 parts of autoclaved deionized water. Mix and use.

Note: Buffer may precipitate and form clear crystals at low temperature. Bring the buffer to room temperature to dissolve any precipitate before use.

Quality control assays :

Analysis	Specification
Appearance	Clear Colourless liquid
pH@25°C (1X Concentration)	7.2±0.2

Ordering Information:

Cat. No	PI No.	Product Description
3101580101730	ELR7	10 X PBS, pH 7.2, 1000 ml

10X ELISA Blocking buffer (BSA)

Description :

ELISA Blocking Buffer is formulated using Phosphate Buffered Saline (PBS) containing 0.02% Sodium azide as preservative along with Bovine Serum Albumin (BSA). It aids in minimizing non-specific reactions in Western Blotting, ELISA and immunohistochemistry-based protocols.

Storage: 4°C:

Note:
- DO NOT REUSE Blocking Buffer.
- The blocking buffer is most compatible with high protein binding ELISA plates.
- For blotting membranes, immerse membranes completely for 2-4 hours at room temperature or overnight at 4° C.

Ordering Information:

Cat. No	PI No.	Product Description
3200981001730	ELR9	10X ELISA Blocking buffer (BSA), 100 ml

ELISA Blocking Buffer (Casein)

Description :

ELISA Blocking Buffer is formulated using Phosphate Buffered Saline (PBS) containing 0.02% Sodium azide as preservative along with Casein powder (supplied separately). It aids in minimizing non specific reactions in Western Blotting, ELISA and immunohistochemistry-based protocols.

Storage: 4°C

Note:
- DO NOT REUSE Blocking Buffer.
- Add Casein powder just before use.
- To increase the solubility add 1 N NaOH drop wise if required.
- The blocking buffer is most compatible with high protein binding ELISA plates.
- For blotting membranes, immerse membranes completely for 2-4 hours at room temperature or overnight at 4° C.

Ordering Information:

Cat. No	PI No.	Product Description
3200481001730	ELR4	ELISA Blocking Buffer (Casein), 100 ml

2X ELISA Blocking Buffer (Gelatin)

Description :

ELISA Blocking Buffer is formulated using Phosphate Buffered Saline (PBS) containing 0.02% Sodium azide as preservative along with Gelatin powder (supplied separately). It aids in minimizing non specific reactions in Western Blotting, ELISA and immunohistochemistry-based protocols.

Storage: Room Temperature

Note:
- DO NOT REUSE Blocking Buffer
- The blocking buffer is most compatible with high protein binding ELISA plates.
- For blotting membranes, immerse membranes completely for 2-4 hours at room temperature or overnight at 4° C.

Ordering Information:

Cat. No	PI No.	Product Description
3200681001730	ELR6	2X ELISA Blocking Buffer (Gelatin), 100 ml

5X Blot Transfer Buffer

Description :

The standard Transfer Buffer for Western Blots, called Towbin buffer which contains 25mM Tris, 192 mM Glycine, pH 8.3 with 5% methanol and 0.1% SDS. This Transfer Buffer has both low ionic strength and low conductivity, which is optimal for tank wet blotting and for semi dry apparatus. Blot Transfer Buffer is supplied at 5X concentration.

Storage: Room Temperature

Quality control assays :

- Performance Test: Protein Molecular Weight Marker, Medium Range (PMWM) was separated on 12% SDS-PAGE, were blotted on to Nitrocellulose Membrane using 1X Blot Transfer Buffer. 90-95 % of Transfer achieved for Proteins between 14.3 kDa - 97.4 kDa which were detected as red color band after staining for 5-10 minutes in Ponceau S Stain followed by destaining with water.

Ordering Information:

Cat. No	PI No.	Product Description
3103585001730	ER35	5X Blot Transfer Buffer, 500 ml

Ezee blue Gel Stainer (No destaining required)

Description :

Ezee Blue Gel Stainer is a ready-to-use brilliant blue G-250 based protein stain for 'one-step' detection of protein bands on polyacrylamide gels. The staining is done in 30 to 60 minutes and no destaining is required. Fixing is also not required prior to staining. Ezee Blue Gel Stainer stains only proteins with relatively clean background, so protein bands are seen during staining. An optional destaining with water can be done to improve the sensitivity.

Storage: Room Temperature

- Do not store in the refrigerator.

Quality control assays :

- Performance Test: 12% SDS-PAGE was prepared with acrylamide/bisacrylamide solution and Protein Molecular Weight Marker, Medium Range (PMWM) was electrophoresed. Sharp, well separated protein bands of PMWM were observed on staining with Ezee Blue Gel Stainer.

Ordering Information:

Cat. No	PI No.	Product Description
3102485001730	ER24	Ezee blue Gel Stainer (No destaining required), 500 ml

Ready to Use Ponceau-S Stain

Description :

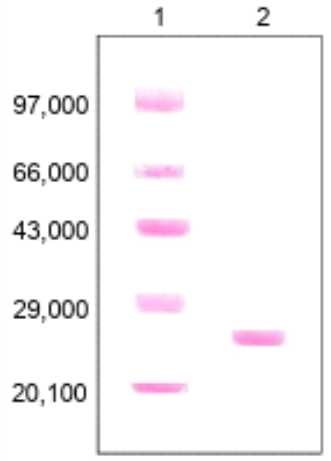
The ready-to-use Ponceau S stain is for rapid and reversible detection of protein bands transferred on to nitrocellulose, PVDF and cellulose acetate membranes. Staining can be done in 5-10 minutes following which lanes could be cut out and probed with different antibodies. Destaining is achieved by rinsing the membrane strips with excess of 1X PBS. The stain is highly sensitive and can detect even low molecular weight proteins and peptides transferred onto nitrocellulose membrane. The resultant red bands can be photographed upon fixation.

Storage: Room Temperature

- Do not store in the refrigerator.

Quality control assays :

- Proteins separated on SDS-PAGE were blotted onto nitrocellulose membrane. The membrane was stained for 5-10 minutes in Ponceau S. The protein bands appeared red in colour on the membrane when rinsed with 1X PBS.



PMW-M and Protein stained with Ponceau-S and fixed with Ponceau-S fixative

Ordering Information:

Cat. No	PI No.	Product Description
2652982501730	ER29	Ready to Use Ponceau-S Stain, 250 ml

Protein A - CL Agarose

Description :

Purified Protien A is covalently coupled to crosslinked beaded agarose by cyanogen bromide activation method. The product is supplied as 1:1 suspension in 10mM sodium phosphate, pH 7.2 with 0.02% sodium azide. Specifications:

Storage: 4° C · Do not freeze.

Quality control assays :

- Performance Test: The binding capacity of Protein A CL-Agarose for human IgG was determined as follows.

Column packed	: 0.5 ml
Normal human serum loaded	: 2 ml
Binding buffer	: 20 mM phosphate buffer pH 7.2
Binding buffer volume	: 5 ml (10 bed volumes)
Wash buffer	: 20 mM phosphate buffer pH 7.2
Wash buffer volume	: 12.5 ml (25 bed volumes)
Elution buffer	: 0.1 M glycine buffer pH 3.0
Elution buffer volume	: 5 ml (10 bed volumes), 1 ml fractions

Ordering Information:

Cat. No	PI No.	Product Description
2110180011730	LIA1S	Protein A - CL Agarose, 1 ml
2110180051730	LIA1M	Protein A - CL Agarose, 5 ml

Glutathione - CL Agarose

Purification of Glutathione – S Transferase (GST) fusion proteins using glutathione-agarose beads is well documented (*). Glutathione is immobilized on cross-linked, beaded 4% agarose by epoxy method using a 10-atom spacer. GST fusion proteins require only single step purification with Glutathione – CL Agarose column. Cellular lysate is passed over the column and the bound GST fusion protein is eluted using a buffer containing reduced glutathione.

Column packed	: 1.0 ml
Clarified GST lysate loaded	: 10 ml
Binding buffer	: 15 mM Sodium phosphate, pH 7.4 and 0.15 M NaCl.
Binding buffer volume	: 5 ml (5 bed volumes)
Wash buffer	: 15 mM Sodium phosphate, pH 7.4 and 0.15 M NaCl.
Wash buffer volume	: 20 - 25 ml (20 – 25 bed volumes)
Elution buffer	: 50 mM Tris, pH 8.0 containing 5 mM reduced glutathione.
Elution buffer volume	: 10 ml (10 bed volumes). 1 ml fractions

References :

- Frangioni, John V. and Benjamin G. Neel (1993). Solubilization and purification of enzymatically active glutathione s-transferase (pGEX) fusion proteins. Anal. Biochem. 210, 179-187.
- Simons, Peter C. and David L.VanderJagt (1977). Purification of gluathione S-transferases for human liver by glutathione-affinity chromatography. Anal. Biochem. 82, 334-341.

Ordering Information:

Cat. No	PI No.	Product Description
2110480051730	LIA35	Glutathione - CL Agarose, 5 ml

Nickel - CL Agarose

Description :

Metal chelate affinity chromatography is gaining wide spread popularity for its use in protein purification. In addition, the technique has been used in analytical applications involving metal ion transfer between proteins,1 peptide mapping and amino acid analysis2 and studies of protein surface structure.3 The development of vectors that allow the expression of 6xHis fusion proteins has lead to the use of immobilized nickel (Ni+2) and cobalt (Co+2) columns in the purification of these recombinant fusion proteins.

The immobilized metals interact with proteins primarily through surface histidine residues. Another example of very strong metal ion/group-specific interaction is that between ferric iron (Fe+3) and compounds that contain phosphate groups.5 In general, it is best to perform the chromatography in the presence of a high ionic strength (0.5-1.0 M NaCl) binding buffer. This condition works to minimize nonspecific electrostatic interaction between the metal ion and the charged proteins in

the sample. When little is known about the protein of interest, immobilized copper (Cu+2) is a good metal ion to try in an initial purification protocol. If the protein is difficult to elute under non-denaturing conditions, other metal ions can be utilized.

Storage: 4° C Do not freeze

Binding capacity of Nickel - CL Agarose was determined as follows:

1. Matrix : Cross linked 4% beaded agarose
2. Ligand : Nickel
3. Coupling : Chelaed through immobilized ininodiacetic acid
4. Column paked : 1.0 ml.
5. Load : Fusion Protein
6. Binding buffer : 500 mM NaC1, 200 mM Sodium Po4 (pH7.0)
7. Binding vol : 5-10 (B.V)
8. Wash buffer : Same as Binding buffer at 20 m Imidazole
9. Wash buffer : 20-25 B.V-20 ml
10. Elution Buffer: 200 mM EDTA Imidazole
11. Elution Buffer vol: 10 BV (10 ml) 1 ml fraction
12. pH Stability : 3-10
13. Storage : In distilled water with 0.5% sodium azide

References :

- Muszynska, G., Zheo, Y.-J. and Porath, J. (1986). Carboxypeptidase A: a model for studying the interaction of proteins with immobilized metal ions. J. Inorg. Biochem. 26, 127-135.
- Yip, T.T., Nakagawa, Y. and Porath, J. (1989). Evaluation of the interaction of peptides with Cu(II), Ni(II), and Zn(II) by high-performance immobilized metal ion affinity chromatography. Anal. Biochem. 183, 159-171.
- Hemdan, E.S., Zhao, Y.-J., Sulkowski, J. and Porath, J. (1989). Surface topography of histidine residues: a facile probe by immobilized metal ion affinity chromatography. Proc. Natl. Acad. Sci. USA 86, 1811-1855.
- Hochuli, E., Dobeli, H. and Schacher, A. (1987). New metal chelate adsorbent selective for proteins and peptides containing neighbouring histidine residues. J. Chromatogr. 411, 177-184.
- Andersson, L. and Porath, J. (1986). Isolation of phosphoproteins by immobilized metal (Fe +3) affinity chromatography. Anal. Biochem. 154, 250-254.

Ordering Information:

Cat. No	PI No.	Product Description
2110580051730	LIA37	Nickel - CL Agarose, 5 ml

Protein A - CL Agarose Pre-packed column

Description :

Purification of immunoglobulins from antisera, ascites fluid or normal sera that can binds to Protein A. 1-6 The Column Genei's prepacked columns are made of inert polystyrene, which is biocompatible, and non-reactive with biomolecules. The affinity matrices are packed in the column with top and bottom porous frits which allow free flow of solvents and prevents column getting dry when not in use. The columns can be used without any appliances by manually adding the sample or buffers. Affinity matrix Genei's, prepacked column, Catalogue # PC201 is packed with 2 ml cross-linked 6% beaded agarose coupled to Protein A. The column provides simple and easy method for isolation of immunoglobulin-G fraction from biological fluids. Protein-A immobilized gel matrix has been widely used for the purification antibodies and normal immunoglobulins.

Column Characteristics

1. Bed Volume : 2 ml
2. Column Size (dia. X height) : 8mm X 45mm
3. Matrix : Cross Linked 6% beaded agarose
4. Ligand : Protein A
5. Coupling : By CNBr method
6. Ligand Amont : 4.4 mg/ml drained gel.
7. Binding Capacity : 13.1 mg/ml drained gel.
8. pH Stabillty : 5 - 10
9. Stoge : At neutral pH with 0.05% sodium azide

References :

- Ey P.L. et al, (1978) Biochemistry 15: 429-436.
- Richman D.D. et al, (1982) J. Immunol 128: 2300-2305.
- Lindmark R. et al, (1983) J. Immunol Method 62: 1-13.
- Jungbauer A. et al, (1989) J. Chrom. 476: 257-268.
- Hermanson, G.T. et al, (1992) immobilized affinity Techniques, Academic, San Diego, CA.
- Kerr M.A. et al, (1994) in immunochemistry lab fax Scientific, Oxford, U.K. pp 83-114.

Ordering Information:

Cat. No	PI No.	Product Description
2120100011730	PC201	Protein A - CL Agarose Pre-packed column, 2 ml

Desalting Column

Description :

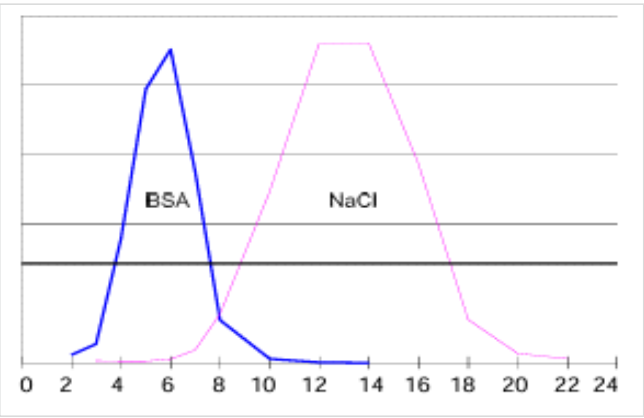
The Desalting columns are prepared by packing size exclusion matrix. The matrix is made of beads of cross-linked dextran with epichlorohydrin. The matrix allows excellent resolution with good flow rates. The fractionation range for globular proteins is between 1,000 – 5,000 Da, with an exclusion limit of approximately 5,000 Da. This ensures group separations of proteins or peptides larger than 5,000 Da, from those with a molecular weight less than 1,000 Da. Desalting column can be used with aqueous solutions in the pH range 2-12. It is stable with all commonly used buffers, solutions of urea (8M), Guanidine hydrochloride (6M), and all non-ionic and ionic detergents. Lower alcohols (methanol, ethanol, propanol) may be used in the buffer or the sample, but we recommend that the concentration be kept below 20% (v/v).

Desalting columns are pre-packed, ready-to-use columns for group separation of high and low molecular weight proteins and peptides.

Features

- Suitable for buffer exchange before or after different chromatographic steps,
- Removal of low molecular weight contaminants and Removal of reagents used to terminate a reaction

Profile of BSA and NaCl on Ds03 column



2 ml BSA 10 mg/ml in 1 M NaCl loaded on 10 ml column and 1 ml fractions collected

References :

- J. Porath and P Flodin, Gel Filtration: A method for desalting and group separation. Nature, 183 (1959)1657– 1659.
- Gel Filtration: Principles and Methods, 7th edition, Pharmacia Biotech, 1997.
- L. Hagel, (1989) Gel Filtration in Protein Purification, Principles, High Resolution Methods, and Applications. J.- C. Janson and L. Rydén (editors), VCH Publishers Inc., New

Ordering Information:

Cat. No	PI No.	Product Description
2122300041730	DS03	Desalting Column (10 ml), 4 Nos

CD4 Monoclonal Antibody

Description :

The CD4 antigen is involved in the recognition of MHC class II molecules and is a co-receptor for HIV. CD4 is primarily expressed in a subset of T-lymphocytes, also referred to as T helper cells, but may also be expressed by other cells in the immune system, such as monocytes, macrophages, and dendritic cells. At the tissue level, CD4 expression may be detected in thymus, lymph nodes, tonsils, and spleen, and in specific regions of the brain, gut, and other non-lymphoid tissues. CD4 functions to initiate or augment the early phase of T-cell activation through its association with the T-cell receptor complex and protein tyrosine kinase, Lck. It may also function as an important mediator of direct neuronal damage in infectious and immune-mediated diseases of the central nervous system. Multiple alternatively spliced transcripts have been identified in this gene.

Applications :

Antibodies that detect CD4 can be used in several scientific applications, including Flow Cytometry, Immunohistochemistry, Western Blot, Immunocytochemistry and Immunoprecipitation. These antibodies target CD4 in Human, Mouse, Rat, Canine and Avian samples. These antibodies have been verified by Relative expression and Cell treatment to confirm specificity to CD4.

Western blot titer: 1:10 – 1:100

Western blot analysis of CD4 was performed by loading 20 µg of Jurkat and CTLL cell lysates onto an SDS polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked at 4ºC overnight. The membrane was probed with a CD4 monoclonal at a dilution of 1:20 overnight at 4° C, washed in TBST, and probed with an HRP-conjugated secondary antibody for 1 hr at room temperature in the dark. Chemiluminescent detection was performed using ECL Western Blotting Substrate.

Store: -20°C

- Store in smaller aliquots to avoid repeated freeze-thaw cycles.

Ordering Information:

Cat. No	PI No.	Product Description
3110492501730	MAB1	CD4 Monoclonal Antibody, 200µl

Polyclonal Antibodies

Introduction :

Antibodies to human and animal whole serum are developed in rabbit and goat. The antibodies are evaluated by IEP and ODD. These antibodies are used in immunological techniques as controls and in preparation of immunological reagents. The antibodies are presented as whole serum.

Nomenclature of polyclonal antibody in this catalogue follows the syntax "Host anti antigen". Thus "Rabbit anti human IgG" refers to antibody against "Human IgG" raised in Rabbit. Genei's polyclonal antibodies are raised against pure antigen using carefully selected animals and optimal long-term immunization protocol. The antibodies are raised in rabbit or goat and isolated from serum.

Quality control assays :

- Quantitative Precipitin Assay: Antibody concentration in the serum was determined by quantitative precipitin assay.
- Immunoelectrophoresis: Specificity of the antiserum to human IgG was determined by Immunoelectrophoresis using human serum.

Rabbit anti human IgG (whole serum)

The antiserum is developed in rabbit using IgG isolated from normal human serum as the immunogen. The antiserum is filter sterilized and frozen.

Storage: -20°C

- Store in smaller aliquots to avoid repeated freeze-thaw cycles

Quality control assays :

- Ouchterlony double diffusion: The presence of Rabbit Anti-human IgG was confirmed by ODD.
- Immunoelectrophoresis: Specificity of the antiserum to human IgG was determined by Immunoelectrophoresis using human serum.

Goat anti mouse IgG (whole serum)

The antiserum is developed in goat using IgG isolated from normal mouse serum as the immunogen. The antiserum is filter sterilized and frozen.

Storage: -20°C Store in smaller aliquots to avoid repeated freeze-thaw cycles

Quality control assays :

- **Ouchterlony double diffusion:** The presence of goat anti mouse IgG was confirmed by
- **Immunoelectrophoresis:** Specificity of the antiserum to mouse IgG was determined by immunoelectrophoresis using mouse serum.

Rabbit anti human serum (whole serum)

The antiserum is developed in rabbit using normal human serum as the immunogen. The antiserum is filter sterilized and frozen.

Storage: -20°C Store in smaller aliquots to avoid repeated freeze-thaw cycles.

Quality Control Assay :

- **Immunoelectrophoresis:** Presence of antibody to human serum proteins in the antiserum was checked by Immunoelectrophoresis using normal human serum.
- Multiple arcs of precipitin lines seen for albumin, IgG and IgM.

Ordering Information:

Cat. No	PI No.	Product Description
0600380051730	AS3M	Goat anti-human IgG (whole serum), 5 ml
0600280051730	AS2M	Rabbit anti-human IgG (whole serum), 5 ml
0600580051730	AS5M	Goat anti-rabbit IgG (whole serum), 5 ml
0600780051730	AS7M	Rabbit anti-bovine IgG (whole serum), 5 ml
0600680251730	AS6L	Goat anti-mouse IgG (whole serum), 25 ml
0601280051730	AS22S	Rabbit anti human serum, 5 ml

Guinea Pig Serum

Description :

Guinea Pig Serum are obtained from non-haemolyzed blood that is collected from healthy animals. The blood is centrifuged, and the serum is collected. This product was aseptically filtered through a $_{0.22}$-micron filter into clean, pre-sterilized containers. And supplied in frozen condition. Each manufactured batch is rigorously controlled, from the collection of serum and throughout all stages of its treatment and production through to final packaging on our premises.

Applications :

Guinea pig serum is suitable blocking agent and negative control in immunoassays. Guinea Pig Serum is used as a supplement to cell culture media. Guinea Pig Serum is also suitable for use as a component of bioassays, immunoassays, or enzyme assays. Guinea Pig Serum provides a broad spectrum of macromolecules, carrier proteins for lipoid substances and trace elements, attachment and spreading factors, low molecular weight nutrients, and hormones and growth factors that promote cell growth and health. These serums provide excellent growth promoting components for tissue culture and microbiological organisms. Guinea Pig Serum is ideal for investigators in Cancer, Immunology, and Cell Biology research.

Store: -$_{20}$°C

- Store in smaller aliquots to avoid repeated freeze-thaw cycles.

Ordering Information:

Cat. No	PI No.	Product Description
3110482501730	NS2CD4	Guinea Pig Serum, 50ml

Goat Serum

Normal goat serum is prepared from healthy non-immunized goats. Normal serum is filter sterilized, and supplied in frozen condition with 0.05% sodium azide.

Serum is use as blocking agent for Histological staining non-specific antibody binding in tissue and cell staining, and in other applications of antibodies. Serum can be used directly for blocking, or as a constituent of a blocking

Storage: -20°C

- Store in smaller aliquots to avoid repeated freeze-thaw cycles.

Quality control assays :

- Ouchterlony double diffusion (ODD): The specificity of serum was tested by ODD.

Rabbit Serum

Normal Rabbit serum is prepared from healthy non-immunized goats. Normal serum is filter sterilized and supplied in frozen condition with 0.05% sodium azide.

Serum is use as blocking agent for Histological staining non-specific antibody binding in tissue and cell staining, and in other applications of antibodies. Serum can be used directly for blocking, or as a constituent of a blocking

Storage: -20° C

- Store in smaller aliquots to avoid repeated freeze-thaw cycles.

Quality control assays :

Ouchterlony double diffusion (ODD): The specificity of serum was tested by ODD.

References :

- In Vitro Cell Dev Biol Anim. 2017 Jul 19;:null - PubMed
- Proc Soc Exp Biol Med. 1971 Nov;138(2):432-7 - PubMed
- PDA J Pharm Sci Technol. 2010 Sep-Oct;64(5):458-64 - PubMed
- Biologicals. 2010 Mar;38(2):211-3 - PubMed

Ordering Information:

Cat. No	PI No.	Product Description
1630180101730	NS1	Goat Serum, 10 ml
1630380101730	NS3	Rabbit Serum, 10 ml

Immunoglobulin-G

Immunoglobulin-G (G-IgG) is fractionated from normal serum by ammonium sulfate precipitation and further purified by an ion exchange chromatography column.

IgG was constituted in 0.01M sodium phosphate pH 7.2 and 0.15M NaCl. IgG is supplied as lyophilized powder.

Storage: 4° C prior to reconstitution.

- Reconstitute with 1 ml of sterile deionized water.

Storage: -20° C. After reconstitution.

- Store in smaller aliquots to avoid repeated freeze-thaw cycles.

Quality control assays :

- Ouchterlony Double Diffusion: Identity of -IgG was confirmed by ODD test.
- SDS-PAGE: The purity of IgG by SDS-PAGE.
- Immunoelectrophoresis: Specificity of IgG was determined by immunoelectrophoresis

Ordering Information:

Cat. No	PI No.	Product Description
1620180101730	IGP1	Goat IgG, 10mg
1620280101730	IGP2	Human IgG, 10mg
1620380011730	IGP3	Mouse IgG, 1mg
1620480101730	IGP4	Rabbit IgG, 10mg
1620580101730	IGP5	Bovine IgG, 10mg

Affinity Purified IgG

Antiserum to Animal immunglobulin-G (IgG) is developed in Animal-2 using purified Animal- 1 as the immunogen. Antibody, specific to Animal-1 IgG is isolated from the antiserum using IgG from affinity column, Animal-1 IgG CL agarose.

The antibody is supplied as a solution in 10 mM sodium phosphate (pH 7.2), 150 mM sodium chloride.

Storage: -20° C

Note: Store in smaller aliquots to avoid repeated freeze – thaw cycles

Quality control assays :

- SDS-PAGE: The purity of antibody confirmed by SDS-PAGE.

Immunoelectrophoresis:

- Specificity of the affinity purified anitibody was determined by immunoelectrophoresis using normal serum. A single precipitation arc was seen.

Ordering Information:

Cat. No	PI No.	Product Description
0610180051730	AA1M	Rabbit-anti goat IgG (Affinity Purified), 5mg
0610280051730	AA2M	Rabbit-anti human IgG (Affinity Purified), 5mg
0610380051730	AA3M	Goat-anti human IgG (Affinity Purified), 5 mg
0610580051730	AA5M	Goat-anti rabbit IgG (Affinity Purified), 5 mg
0610680051730	AA6M	Goat-anti mouse IgG (Affinity Purified), 5 mg
0610780051730	AA7M	Rabbit anti Bovine IgG (Affinity Purified), 5mg

Alkaline Phosphatase Conjugates

Antibody specific to Animal-1 IgG is isolated by affinity purification from the antiserum developed in Animal -2. The affinity isolated antibody is then conjugated with ALP, by maleimide method. 0.1% sodium azide is added as preservative.

Specification: Antibody concentration 0.5-1.5 mg/ml

Quality control assays :

- Direct ELISA (dELISA): Microtiter plates were coated with purified Animal IgG. 100 µl of diluted ALP conjugate was allowed to bind for 30 minutes at 37° C. Concentration of bound conjugate was estimated directly using p-nitro phenyl phosphate, 1 mg/ml in 1 M diethanolamine pH 9.8. The dilution which corresponds to a reading of 1.0 to 1.2 at 405 nm in ELISA reader is quoted as titer
- Western Blotting: Confirmed by Direct or Indirect ELISA

Ordering Information:

Cat. No	PI No.	Product Description
1100180011730	ALP1M	Goat anti-rabbit IgG - ALP, 1 ml
1100280011730	ALP2M	Goat anti-human IgG - ALP, 1 ml
1100480011730	ALP4M	Goat anti-mouse IgG - ALP, 1 ml
1100680011730	ALP6M	Rabbit anti-goat IgG - ALP, 1 ml
1100980011730	ALP9M	Rabbit anti-mouse IgG - ALP, 1 ml

Streptavidin - ALP

Streptavidin is conjugated with alkaline phosphatase, by maleimide method. 0.1% sodium azide is added as preservative. Specification: Activity of streptavidin 15 Units/mg. Protein concentration 1 mg/ml

Storage: 4° C

Do not freeze

Directions to use: Working dilution will depend on assay condition. Due to differences in assay systems, these titers may not reflect the user's actual working dilution.

Quality control assays :

- Indirect ELISA (dELISA): Microtiter plates were coated with purified Rabbit IgG at 5 µg/ml concentration (0.5 µg/100 µl) in 0.05M carbonate-bicarbonate buffer pH 9.6. Streptavidin ALP conjugate indirectly titrated by allowing to bind 100 µl of 1:10,000 diluted Goat anti Rabbit IgG biotin conjugate at 37° C for 30 minutes. Different dilution of streptavidin ALP conjugate was then added and incubated for 15 minutes at 37° C. ALP conjugate was estimated using p-nitro phenyl phosphate having concentration of 1 mg/ml in 1M diethanolamine pH 9.8. The dilution which corresponds to a reading of 1.0 to 1.2 at 405 nm in ELISA reader is quoted as titer. Indirect ELISA titer is 1:10000
- Western Blotting: Rabbit IgG detected indirectly

using 10 µg of protein under reducing conditions on SDS-PAGE. The protein was then transferred to nitrocellulose membrane, blocked with 1% ovalbumin, incubated with Goat anti Rabbit IgG biotin conjugate 1:5000 diluted). Membrane was washed and incubated with streptavidin ALP conjugate and blot developed with BCIP/NBT (SFE2). Western Blot titer is 1:15000

Ordering Information:

Cat. No	PI No.	Product Description
1100575001730	ALP5	Streptavidin - ALP, 0.5 ml

GOLD CONJUGATE

Protein A Gold Conjugate

Staphylococcal Protein A contains five high-affinity binding sites that are capable of interacting with Fc region from IgG of several species. Protein A is affinity-isolated and covalently coupled to Colloidal Gold to produce Protein A Gold conjugate, which can be used in detection systems such as immunoblotting- western or dot blot.

Storage: 4° C

Quality control assays :

- Performance Test: Dot Blot

Ordering Information:

Cat. No	PI No.	Product Description
1130480011730	Ga04	Protein A Gold Conjugate

Rabbit anti bovine IgG Gold Conjugate

Antibody specific to Bovine IgG is purified by affinity purification from the antiserum developed in rabbit. The antibody is then conjugated with Colloidal Gold, which can be used in detection systems such as immunoblotting western or dot blot. Specification:

Storage: 4° C

Quality control assays :

- Performance Test: Dot Blot

Ordering Information:

Cat. No	PI No.	Product Description
1130780011730	GA07	Rabbit anti bovine IgG Gold Conjugate

FLUORESCEIN CONJUGATES

Fluorescein and tetramethylrhodamine conjugates to protein or dextran were used to determine subcellular pH. The pH dependence of fluorescence of fluorescein isothiocyanate (FITC) conjugates could be described by a single proton dissociation (pKa' ~6.8). This allowed pH to be derived accurately from spectra using the simple Henderson-Hasslebach equation

LABEL	FITC
Excitation Maximum nm	494
Emission Maximum nm	520
Conjugation method	Thiocyanate
Molar Incorporation	2-5
Antibody or protein	1-1.2 mg/ml
Storage	4-C
Titre	PIFA 1:40 to 1:80
Applications	Immunohistochemistry Immunocytochemistry

- Antibody specific to Source-1 IgG is isolated by affinity purification from the antiserum developed in Source-2. The affinity isolated antibody is then conjugated with fluorescein isothiocyanate.
- **Specification:** Fluorescein to antibody molar ratio is definedAntibody concentration

Storage: 4° C

Note:
- For continuous use, keep the product refrigerated.
- For extended use, the product can be stored frozen in smaller aliquots.
- Repeated freeze-thaw cycles not recommended.

Ordering Information:

Cat. No	PI No.	Product Description
1120180011730	FTC1	Goat anti human IgG - FITC, 1ml
1120280011730	FTC2	Goat anti rabbit IgG - FITC, 1 ml
1120380011730	FTC3	Goat anti mouse IgG - FITC, 1 ml
1120480011730	FTC4	Rabbit anti goat IgG - FITC, 1ml
1120580011730	FTC5	Rabbit anti mouse IgG - FITC, 1ml

RHODAMINE CONJUGATES

Rhodamine 110 is a xanthene dye first synthesized by Maurice Ceresole over a century ago . Its fluorescence is bright and pH-insensitive, and its emission and excitation wavelengths are ideal for biological assays . To adapt rhodamine 110 as a reporter of enzymatic catalysis, we have employed the “trimethyl lock” as a trigger that couples fluorescence generation to a designated chemical reaction . The trimethyl lock is an o-hydroxydihydrocinnamic acid derivative in which steric interactions between three methyl groups leads to rapid lactonization to a dihydrocoumarin ring with concomitant release of an alcohol or amine.

The isothiocyanate derivative of the fluorophore, fluorescein, or rhodamine, is coupled to the amino groups of IgG antibody in a one-step procedure and excess label is removed by gel filtration.

Storage: 4°C

Ordering Information:

Cat. No	PI No.	Product Description
1150180011730	RTC1	Goat anti human IgG - TRITC, 1ml
1120680011730	RTC2	Goat anti rabbit IgG - TRITC, 1ml
1150380011730	RTC3	Goat anti mouse IgG - TRITC, 1ml
1120780011730	RTC4	Rabbit anti goat IgG - TRITC, 1ml
1120880011730	RTC5	Rabbit anti mouse IgG - TRITC, 1ml

PEROXIDASE CONJUGATES

Horseradish peroxidase (HRP) is an enzyme used to amplify signal in photometric assays by catalyzing the conversion of chromogenic or chemiluminescent substrates for the detection of targets such as proteins, carbohydrates, and nucleic acids.

Antibody specific to Source-1 IgG is isolated by affinity purification from the antiserum developed in Source-2. The affinity isolated antibody is then conjugated with horseradish peroxidase, by periodate method.

Ordering Information:

Cat. No	PI No.	Product Description
1140180011730	HPO1	Rabbit anti-human IgG - HRP, 1 ml
1140280011730	HPO2	Goat anti-human IgG - HRP, 1 ml
1140380011730	HPO3	Goat anti-rabbit IgG - HRP, 1 ml
1140480011730	HPO4	Rabbit anti-goat IgG - HRP, 1 ml
1140580011730	HPO5	Rabbit anti-mouse IgG - HRP, 1 ml
1140680011730	HPO6	Goat anti-mouse IgG - HRP, 1 ml
1140975001730	HPO9	Streptavidin - HRP, 0.5 ml
1141380011730	HPO13	Rabbit anti horse IgG - HRP, 1 ml
1141480011730	HPO14	Rabbit anti rat IgG - HRP, 1 ml

1142075001730	HPO20	Goat anti-rabbit IgG - HRP, Adsorbed with Bovine, Human, Mouse Serum Proteins (0.5ml)
1142175001730	HPO21	Goat anti-mouse IgG - HRP Adsorbed with Bovine, Human, Rabbit Serum Proteins (0.5ml)

ENZYME FOR ELISA

Alkaline phosphatase

Description :

Alkaline phosphatase serves as both a model enzyme for studies on the mechanism and kinetics of phosphomonoesterases and as a reporter in enzyme-linked immunosorbent assays (ELISAs) and other biochemical methods. The tight binding of the enzyme to its inorganic phosphate product leads to strong inhibition of catalysis and confounds measurements of alkaline phosphatase activity.

- Source : Bovine intestinal mucosa.
- Storage Solution : A solution in 50% glycerol containing 5 mM Tris (pH ~ 7.0), 0.005 M MgCl2 and 0.1 mM zinc chloride.
- **Unit Definition : 1** unit of enzyme is defined as the amount of enzyme required to hydrolyze 1 micromole of 4-nitro phenyl phosphate in 1 minute at 37°C in 0.9 ml of 1M diethanolamine buffer pH 9.8, 0.25mM $MgCl_2$.
- **Specific Activity :** Lot Specific
- **Protein Concentration :** Lot Specific

Storage : 4° C, Do not freeze.

Ordering Information:

Cat. No	PI No.	Product Description
1650180051730	EE1L	Alkaline phosphatase, 5 mg

SUBSTRATES FOR ELISA

TMB/H_2O_2 for ELISA (20 X Conc.)

Description :

TMB/H_2O_2 is chromogenic substrate for enzyme Horseradish Peroxidase (HRP). TMB/H_2O_2 (Tetramethyl benzidine/Hydrogen peroxide) produces a soluble blue color with HRP. This reaction can be stopped with equal volume of 1N sulfuric acid. The optical density of resulting yellow color can be read at 450nm. TMB is reported to be non-carcinogenic and more sensitive than O-phenylenediamine dihydrochloride (OPD).

Storage: 4° C.

Quality control assays :

- pH Check: pH of 1X concentration is 4.5
- Color: 20X TMB/H_2O_2 appears as clear yellow solution.
- Performance Test: 1X TMB/H_2O_2 is tested in ELISA to detect Horse radish peroxidase activity which yields a blue colour that changes to yellow (Amax = 450 nm) upon addition of 1N H_2SO_4 stop solution.

Applications :

- Enzyme-Linked Immunosorbent Assay (ELISA): TMB/H_2O_2 is widely used in ELISA for the detection of antigens or antibodies. The reaction between peroxidase-conjugated antibodies or antigens and TMB/H_2O_2 produces a colored product, and the intensity of the color is proportional to the amount of bound antibody or antigen.
- Enzyme Activity Assays: TMB/H_2O_2 is used in assays to detect and quantify the activity of peroxidase enzymes in biological samples. The color change is indicative of the enzymatic reaction and can be measured spectrophotometrically for quantitative analysis.
- Colorimetric Assays: It is used in various plate-

based assays, such as colorimetric assays for detecting enzyme activities, quantifying biomolecules, or screening for specific analytes.

Ordering Information:

Cat. No	PI No.	Product Description
1610180101730	SFE1	TMB/H_2O_2 for ELISA (20 X Conc.), 10 ml

BCIP/NBT

Description :

BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) is a commonly used substrate for use with alkaline phosphatase. The reaction results in the formation of an insoluble, colored product, and the intensity of the color is directly proportional to the concentration of the alkaline phosphatase enzyme.

Unique features :

- **Substrate Components:** BCIP (5-bromo-4-chloro-3-indolyl phosphate): A colorless substrate for alkaline phosphatase that produces a blue precipitate upon enzymatic cleavage.
- **NBT (nitro blue tetrazolium):** A yellow tetrazolium salt that reacts with the cleaved BCIP to form an insoluble, bluish-grey to black precipitate.
- **Color Intensity and Enzyme Concentration:** The intensity of the bluish-grey to black color is directly proportional to the concentration of alkaline phosphatase in the sample. This makes the substrate useful for both qualitative detection and quantitative analysis.
- **Readout and Visualization:** The color development can be visually inspected, and the results can be documented by photography or imaging systems. The appearance of a colored band or spot indicates the presence of alkaline phosphatase.
- **Ready-to-Use Solution:** Researchers can directly apply the substrate solution to the membrane or other assay format.
- Substrate Stability: BCIP/NBT solutions are typically light-sensitive and should be stored in the dark, 4°C. Prolonged exposure to light may result in substrate degradation and reduced sensitivity.
- Color Development Control: The color development can be stopped by washing or immersing the membrane or assay in a buffer containing an alkaline phosphatase inhibitor or by using an acidic solution.
- High Sensitivity: BCIP/NBT is known for its high sensitivity, allowing the detection of low levels of alkaline phosphatase activity.

Ordering Information:

Cat. No	PI No.	Product Description
1610280501730	SFE2	BCIP/NBT, 50 ml

TMB/H_2O_2 for Localization

Description :

TMB/H_2O_2(Tetramethyl benzidine/Hydrogen Peroxide) is a chromogenic substrate predominantly useful in development of blots, especially for the detection of Horseradish Peroxidase (HRP) activity.

Features :

- **Chromogenic Substrate:** TMB/H_2O_2 serves as a chromogenic substrate specifically for Horseradish Peroxidase (HRP).
- **Insoluble Reaction Product:** The enzymatic reaction between HRP and TMB/H_2O_2 results in the formation of an insoluble reaction product.
- **Bluish Precipitates:** The insoluble reaction product manifests as bluish precipitates, providing a visual indication of HRP activity.
- **Membrane Applications:** TMB/H_2O_2 is particularly useful in membrane-based applications. In these applications, the bluish precipitates deposit on the immobilized HRP, facilitating detection.

DAB Solution (for HRP) - ELISA

Applications :

- **Western Blotting:** TMB/H_2O_2can be used to visualize proteins in Western blotting. Horseradish peroxidase (HRP)-conjugated secondary antibodies bind to specific proteins, and the subsequent reaction with TMB/H_2O_2 produces a colored signal on the membrane, allowing the detection of target proteins.
- **Immunohistochemistry (IHC) an Immuno -cytochemistry (ICC):** TMB/H_2O_2 is employed for the visualization of antigens in tissue sections (IHC) or cultured cells (ICC). The peroxidase enzyme is often used to label primary or secondary antibodies, and the TMB/H_2O_2 reaction produces a visible signal at the site of the target antigen.
- **Hybridization Assays:** TMB/H_2O_2 can be employed in hybridization assays, where peroxidase-labelled probes are used to detect nucleic acid targets. The reaction produces a visible signal that indicates the presence of the target nucleic acid.
- **Reagent Concentration:** The supplied reagent has a 10X concentration. The reagent is diluted to 1X (1:10) in distilled water just before use. The diluted reagent not recommended to be stored.

Quality control Assay :

- **Performance Test:** Performance of TMB/H2O2 was tested in membrane application for detection of horse radish peroxidase TMB/H2O2

Storage: 4°C.

References :

- Josephy, P. D., Eling, T., and Mason, R. P. (1982) The horseradish peroxidase-catalyzed oxidation of 3,5,32,52-tetramethylbenzidine. Free radical and charge-transfer complex intermediates. J. Biol. Chem. 257, 3669–3675.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Ordering Information:

Cat. No	PI No.	Product Description
1610380101730	SFE3	TMB/H2O2 for l ocalization, (10 X Conc.) 10 ml

Description :

3-3'-Diaminobenzidine (DAB) is the most commonly used chromogen for demonstrating the presence of horseradish peroxidase in immunohistochemistry or immunoblotting. It produces a stable water/alcohol insoluble reddish brown product OR blackish color with enhancer. The stable DAB (50X) concentrate supplied is sufficient for making 100 ml working solution. Appearance: The DAB (50X) concentrate is an amber solution and the working solution is a light pink clear solution. All other solutions are clear.

Features :

- **Commonly Used Chromogen:** 3,3'-Diaminobenzidine (DAB) is a widely utilized chromogen in immunohistochemistry (IHC) and immunoblotting.
- **Detection of Horseradish Peroxidase (HRP):** DAB is specifically employed for demonstrating the presence of Horseradish Peroxidase (HRP) in biological samples.
- **Color Development:** The chromogenic reaction with HRP results in the formation of a stable, water/alcohol-insoluble product. The typical color produced is reddish-brown, providing a clear visual indication.
- **Enhancement for Intensified Color:** In some cases, an enhancer can be used to intensify the color development, leading to a blackish color.
- Stable DAB (50X) Concentrate: The supplied DAB concentrate is at a 50X concentration, offering high efficiency. This concentrate is sufficient for preparing a working solution, making it cost-effective.
- **Working Solution Preparation:** Researchers typically dilute the concentrate to create a working solution suitable for their specific experimental needs.

- **Insoluble Product Formation:** The DAB chromogen produces a water/alcohol-insoluble product, contributing to the stability of the reaction.
- **Colorimetric Signal Stability:** The reddish-brown color or intensified blackish color remains stable over time, allowing for reliable visual interpretation.

Quality control assays :

- **Performance Test:** Performance of 3-3'-diaminobenzidine tetra hydrochloride (DAB) was tested in detection of horseradish peroxidase (HRP).

Storage: 4°C

Applications :

- DAB is a crucial component in immunohistochemical staining procedures, where the visualization of specific antigens is essential.
- It is also employed in immunoblotting techniques for protein detection.
- Due to its versatility, stability, and common usage, DAB is a staple in laboratories performing immunohistochemistry and related applications.

References :

- D.A.B. (Diaminobenzidine)"
- Chesapeake Bay Division, International Association for Identification. Archived from the original on 23 December 2007. Retrieved 2007-11-09

Ordering Information:

Cat. No	PI No.	Product Description
1610900011730	SFE9	DAB (50X), 2 ml

DAB System

Description :

3-3'-diaminobenzidine tetrahydrochloride (DAB) is a substrate for horseradish peroxidase that yields a colored deposit insoluble in aqueous medium, alcohol and xylene. DAB is suitably packed so as to avoid unnecessary handling of DAB by investigator.

DAB should be used immediately after dissolving. Do not store.

- DAB should be used immediately after dissolving. Do not store.

Kit Contents :

DAB system consists of

- DAB
- Dilution Buffer
- H_2O_2

Store: 4°C.

Quality control assay :

Performance Test: Performance of 3-3' – diaminobenzidine tetra hydrochloride (DAB) was tested in detection of horse radish peroxidase (HRP).

Applications :

- **Compatibility with Tissue Processing:** The insolubility of the colored deposit in xylene enhances compatibility with standard tissue processing protocols commonly utilized in histology.
- **Visual Detection Under Microscopy:** The colored deposit formed by DAB facilitates easy visual detection under a microscope, aiding researchers in precisely localizing target antigens within tissue sections.
- **Immunohistochemical Staining:** DAB plays a crucial role as a substrate in the immunohistochemical staining process, enabling the visualization of specific proteins or antigens in tissues.

References :

- D.A.B. (Diaminobenzidine)" Chesapeake Bay Division, International Association for Identification. Archived from the original on 23 December 2007. Retrieved 2007-11-09

Ordering Information:

Cat. No	PI No.	Product Description
1610500011730	SFE5	DAB System (Buffer + Substrate + Chromogen + Metal Enhancer), 10x6mg

BSA (Bovine Serum Albumin)

Description :

BSA or Bovine Serum Albumin, is a protein commonly derived from bovine serum. BSA is a single polypeptide chain consisting of about 583 amino acids with a molecular weight of approximately 68,000 Daltons. It belongs to the albumin family of proteins.

Serum albumin may be referred to as Fraction V. This naming convention is taken from the original Cohn method of fractionating serum proteins using cold ethanol precipitation. Serum albumin was found in the fifth ethanol fraction using Cohn's method.

Serum albumins is purified from a variety of primary methods including the true Cohn fractionation method, modified ethanol fractionation methods, heat shock and chromatography. Additional purification steps may include crystallization or charcoal filtration. BSA(fraction V) is one of the purest forms of BSA available It is extensively purified to remove impurities, contaminants, and other proteins

Applications :

- Bovine Serum Albumin Fraction V can be used in a variety of applications:
- Standard protein for protein estimation Assays.
- Blocking agent of all protein binding sites in ELISAs, Southern blots, and western blots.
- Stabilizer for restriction enzyme buffers
- Media supplement; for media that require addition of protein.
- Carrier protein for peptides in antibody production.
- Molecular weight: 68 kDa

Storage conditions: 4°C

Ordering Information:

Cat. No	PI No.	Product Description
1650500501730	B5	BSA (Bovine Serum Albumin), 50 g

MODIFIED FREUND'S COMPLETE ADJUVANT AND FREUND'S INCOMPLETE ADJUVANT

Modified Freund's Complete Adjuvant (FCA-M)

Description :

Freund's Complete Adjuvant is a popular nonspecific stimulator of the immune response for use in antibody production immunization process.

This product comes in 10 mL ampules that are convenient and ready to use. The water-and-oil emulsion greatly enhances immune responses to immunogens when mixed and injected with the prepared antigen and is typically used for initial injections.

Freund's Complete Adjuvant (FCA), also known as Complete Freund's Adjuvant (CFA), comprises non-metabolizable oils like paraffin and mannide monooleate, and heat killed mycobacteria. These non-metabolizale oils help in formation of water in oil emulsion with aqueous antigen which helps in retention of antigen for longer times at the site of injection and therefore helps in boosting immune response. Furthermore, heat-killed mycobacteria attract macrophages and initiate cell-mediated immune response which is long lasting.

Application :

Antigens are typically mixed with an equal volume of the adjuvant to form an emulsion.

Freund's Adjuvants may be used to produce water-in-oil emulsions of immunogens. Antigens in water-in-oil emulsions stimulate high and long-lasting antibody responses which can be attributed to the slow release of antigen.

The mycobacteria in Complete Freund's adjuvant attracts macrophages and other cells to the injection site which enhances the immune response. For this reason, Complete Freund's Adjuvant is used for initial injections and Incomplete Freund's Adjuvant for subsequent boosts.

Store: Room temperature ($_{15}$°- $_{30}$°C).

Modified Freund's Inomplete Adjuvant (FIA-M)

Description :

Freund's Incomplete Adjuvant is a nonspecific stimulator of the immune response for use in antibody production immunization process. This product comes in $_{10}$ mL ampules that are convenient and ready-to-use. The water-and-oil emulsion greatly enhances immune responses to immunogens when mixed and injected with the prepared antigen and is typically used for booster injections. The ampules comprise conveniently sized aliquots that provide long shelf life.
Freund's Incomplete Adjuvant is a mixture of $_{85}$% paraffin oil and $_{15}$% mannide monooleate. It is used for making emulsion of immunogen. The emulsified immunogen on injecting to any host (animal) causes slow release of immunogen causing high and prolonged antibody response. Freund's Incomplete Adjuvant lacks the mycobacteria found in Complete Freund's Adjuvant thereby minimizing the side-effects.

Application :

Adjuvants are nonspecific stimulators of the immune response. When mixed with an antigen or immunogen, adjuvants help to deposit or sequester the injected material thereby helping to increase antibody response. Adjuvants enhance the immune response to compounds that are already immunogenic; they do not confer immunogenicity to non-immunogenic biomolecules. To make prospective antigens more immunogenic, it is necessary to conjugate them to a carrier protein or some other complex, immunogenic molecule.

References :

- Adjuvant activity of incomplete Freund's adjuvant-FC Jensen 1, JR Savary, JP Diveley, JC Chang
- J. Freund, J. Casals, E.P. Hosmer, Sensitization and antibody formation after injection of tubercle bacilli and paraffin oil, Proc. Soc. Exp. Biol. 37 (1937) 509–513.
- J. Freund, Some aspects of active immunization, Annu. Rev. Microbiol. 1 (1947) 291–308.
- J. Freund, The effect of paraffin oil and mycobacteria on antibody formation and sensitization: a review, Am. J. Clin. Pathol. 21 (1951) 645–656.

Ordering Information:

Cat. No	PI No.	Product Description
1640280401730	FIA-M	Modified Freund's Incomplete Adjuvant, 100ml
1640180401730	FCA-M	Modified Freund's Complete Adjuvant, 100ml

Note: Bulk pack available on request (1ml / 5 ml/ 10 ml)

Albumin depletion kit

Description :

One major challenge in functional proteomics is the separation of complex samples prior to comparative analysis. The best source of proteins for identification of potential disease markers are body fluids such as serum, cerebrospinal fluid, plasma etc., However, the main drawback of a comprehensive analysis of body fluids is the high abundance of serum albumin. Serum albumin can constitute 60-70% of the total serum protein. The concentration of this protein causes loss of resolution of lower abundance proteins difficult in two-dimensional gel electrophoresis (2 DGE).

Depletion of albumin removes approximately 50% of 75% of total serum proteins, so that 3-4 times more enriched sample can be loaded on 2DGE, allowing the visualization and analysis of the remaining proteins easier.

The Genei Albumin Kit provides a fast, reproducible, highly specific and efficient method to deplete albumin from body fluids, such as plasma or serum.

The matrix is a blue dye conjugated to an agarose based resin

Features :

- Efficient removal of albumin - facilitating analysis and visualization of low abundance proteins.
- Easy-to-use spin column format is a fast procedure - saving time and streamlining analysis.
- Can be used for multiple species without protocol change
- Can be used for different samples like serum, plasma and cerebrospinal fluid.
- 80-90% of albumin is removed.

Kit Contents :

- Blue Agarose Suspension
- Equilibration Buffer
- Extraction Buffer
- Spin Columns

Applications :

- Albumin Depleted enriched samples can be loaded on 2 DGE, allowing visualization of proteins of low abundance

References :

- Olver CS, Webb TL, Long LJ, Scherman H, Prenni JE. Vet Clin Pathol. (2010) Sep Comparison of methods for depletion of albumin and IgG from equine serum. 39(3):337-45
- Rengarajan, K. et al., BioTechniques, (1996) Removal of albumin from multiple human serum samples. 20, 30-32

Ordering Information:

Cat. No	PI No.	Product Description
166010001730	KT118	Albumin depletion kit, 5 preps.

Albumin and IgG depletion kit

Description :

One of the major challenges in functional proteomics is the separation of complex samples prior to comparative analysis. The best sources of proteins for identification of potential disease markers are body fluids such as serum, cerebrospinal fluid, plasma, etc. However, the main drawback of a comprehensive analysis of body fluids is the high abundance of serum albumin and IgG. Serum albumin can constitute 60-70% of the total serum protein and IgG 10-25%. The concentration of these proteins may cause loss of resolution of lower abundance proteins in two-dimensional electrophoresis (2DGE). Depletion of Albumin and IgG removes approximately 50-75% of total serum proteins, so that 3-4 times more enriched sample can be loaded on 2DGE, allowing the visualization and analysis of the remaining proteins easy.

The Genei Albumin and IgG Depletion Kit provides a fast, reproducible, highly specific and efficient method to deplete albumin and IgG from body fluids, such as plasma or serum.

The matrix is a mixture of two media:

- A blue dye conjugated to an agarose base resin
- Protein A Agarose for the capture of IgG.

Kit Contents :

- Blue Sepharose and IgG Depletion Medium
- Equilibration Buffer
- Extraction Buffer
- Spin Columns

Features :

- Efficient removal of albumin and IgG, facilitating the analysis and visualization of low abundance proteins.
- Easy to use column format is a fast procedure, saving time and streamlining analysis.

- Can be used for multiple species without protocol change.
- Can be used for different samples like serum, plasma.
- 70 -75% of Albumin and IgG are removed.

References :

- Olver CS, Webb TL, Long LJ, Scherman H, Prenni JE. Vet Clin Pathol. (2010) Sep Comparison of methods for depletion of albumin and IgG from equine serum. 39(3):337-45
- Rengarajan, K. et al., BioTechniques, (1996) Removal of albumin from multiple human serum samples. 20, 30-32

Ordering Information:

Cat. No	PI No.	Product Description
166020001730	KT119	Albumin and IgG depletion kit, 5 preps