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IgG Purification Kit (Protein A Based), 2 ml column

Description :

The cell wall of the organism Staphylococcus aureus contains a protein designated as Protein A, which is a single polypeptide with molecular weight of around 35-50kDa and is almost devoid of any carbohydrates. This protein exhibits the unique and important property of binding to the Fc region of immunoglobulins with a high degree of specificity. It binds especially to IgG of several species thereby concentrating and purifying the IgG free from IgA, IgM and non-immunoglobulin serum proteins such as albumin. Protein A is an extremely stable molecule and can survive high temperatures and chemical agents routinely used in protein chemistry such as 4M urea, thiocyanate, guanidine hydrochloride etc. It serves as a very important medium for protein purification by column affinity chromatography. It is covalently linked to agarose using the cynogen bromide activation method and packed in a column. Affinity chromatography has proved to be a very effective single step tool for protein concentration and purification and gives yields of as much as 95% purity. It should be noted that Protein A binds with varying affinity to the different IgG subclasses. For instance, it binds strongly to human IgG1, IgG2 and IgG4 but not to IgG3 or it binds poorly to mouse IgG1 and a majority of rat immunoglobulins. Monoclonal antibodies too may not bind to Protein A. However, the interaction between Protein A and IgG is salt and pH dependent and therefore those antibodies with poor binding affinity may bind at high salt concentrations of 2-3M NaCl and pH 8-9. High salt concentration reduces the ionic interaction and enhances hydrophobic interactions between Protein A and IgG.

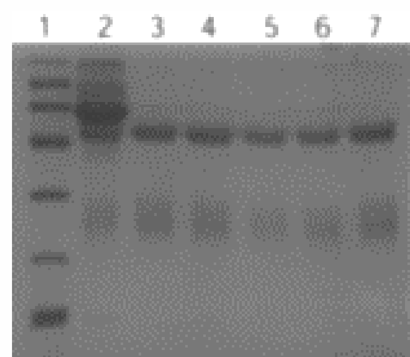
IgG Purification Kit is useful for purification of Immunoglobulin G from serum using Protein A Agarose where Protein A from Staphylococcus aureus is covalently immobilized onto cross linked agarose beads.

Features :

- One step purification.
- Simple, Rapid and convenient protocol.
- High purity of IgG.
- Robust and gentle purification.
- Re-generable support
- Complete kit

Kit Contents :

- Protein A Agarose Column
- Equilibration Buffer (25X)
- 5X Elution Buffer
- Storage Buffer (25X)
- Neutralizing Buffer



Analysis of Rabbit IgG purified 5 times using a regenerated Protein A Agarose Column on 12% SDS-PAGE.

Lane	Description
Lane 1	Protein Molecular Weight Marker (97.4-14 3KD)
Lane 2	Normal Rabbit Serum
Lane 3	I time (1st use)
Lane 4	II time (2nd use)
Lane 5	III time (3rd use)
Lane 6	IV time (4th use)
Lane 7	V time (5th use)

Reference :

- Bjork, L., et al. (1972). Some physicochemical properties of protein A from Staphylococcus aureus. Eur. J. Biochem. 29, 579-584.
- Sjoquist, J., et al. (1972). Protein A isolated from Staphylococcus aureus after digestion with lysostaphin. Eur. J. Biochem. 29, 572-578.
- Hjelm, H., et al. (1975). Immunologically active and structurally similar fragments of protein A from Staphylococcus aureus. Eur. J Biochem. 57, 395-403.
- Sjoholm, I. (1975). Protein A from Staphylococcus aureus. Spectropolarimetric and Spectrophotometric studies. Eur. J. Biochem. 51, 55-61.
- Goding, J.W. (1978). Use of Staphylococcal protein A as an immunological reagent. J. Immunol. Methods 20, 241-253.
- Lindmark, R., Thoren-Tolling, K. and Sjoquist, J. (1983). Binding of Immunoglobulins to protein A and immunoglobulin levels in mammalian sera. J. Immunol. Methods 62, 1-13.
- Kronvall, G. (1970). Phylogenetic insight into evolution of mammalian Fc fragment of gamma G globulin using staphylococcal protein A. J. Immunol. 104, 140-147.
- Kronvall, G. and Williams, R.C. (1969). Differences in anti- protein A activity among IgG subgroups J. Immunol. 103(4), 828-833.
- Surolia, A., et al. (1982). Protein A: Nature's universal anti- body TIBS 7, 74-76.
- Kronvall, G., et al. (1970). Protein A reactivity with mouse immunoglobulins J. Immunol., 105, 1116-1123.
- Skvaril, F. (1976). The question of specificity in binding human IgG subclasses to protein A-Sepharose. Immunochem. 13, 871-872.
- Ey, P.L., Prowse, S.J. and Jenkin, C.R. (1978). Isolation of pure IgG1, IgG2a, and IgG2b immunoglobulins from mouse serum using protein A Sepharose. Immunochem. 15, 429-436

Ordering Information:

Cat. No	PI No.	Product Description
2160100021730	Kt16	IgG Purification Kit (Protein A Based), 2 ml column

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Immunoglobulin Purification Kit (Thiophilic Column)

Description :

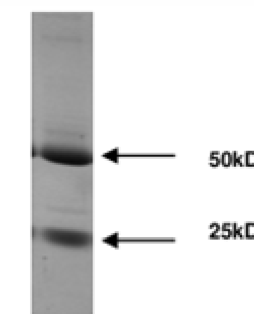
Thiophilic adsorption is useful for the purification of immunoglobulins under mild conditions. Although there are several established procedures for the purification of immunoglobulins, thiophilic adsorption appears thus far to be unique in its capacity to adsorb three major classes of immunoglobulins IgG, IgA and IgM. Furthermore, in contrast to other affinity purification methods, recovery of the adsorbed immunoglobulins from the thiophilic adsorption matrix is accomplished efficiently at neutral pH, without the need for perturbation of protein structure. Immunoglobulin Purification Kit is based on Thiophilic Adsorption Chromatography (TAC). TAC is a group specific, salt-dependent purification technique with distinct adsorption affinity towards immunoglobulins. The term thiophilic refers to an affinity for sulfone group that lies near thio ether groups. TAC as proven to be an easy and economical method for purification of immunoglobulins.

Features :

- Easy purification protocol.
- Mild elution does not cause any adverse effect on purified immunoglobulin.
- This protocol removes 90-95% of albumins from the serum sample.

Kit Contents :

- 2-Mercaptopyridine- CL Agarose Column
- Binding Buffer (5X)
- Elution Buffer (15X)



Analysis of 20 µg of Goat immunoglobulin purified from goat serum using the kit on 12% SDS PAGE.

Reference :

- Hutchens, T. W. and Porath, J. (1986) Thiophilic adsorption of immunoglobulins-Analysis of conditions optimal for selective immobilization and purification. Anal. Biochem. 159, 217-226.
- Nopper, B., Kohen, F., and Wilchek, M. (1989) A thiophilic adsorbent for the one-step high-performance liquid chromatography purification of monoclonal antibodies. Anal. Biochem. 180, 66-71

Ordering Information:

Cat. No	PI No.	Product Description
2160300011730	Kt38	Immunoglobulin Purification Kit (Thiophilic Column), 3 preps

Protein A Immunoprecipitation Kit, 20 preps

Description :

Immuno-precipitation (IP) is the technique of precipitating a protein antigen out of solution using an antibody that specifically binds to that protein. This process can be used to isolate and concentrate a particular protein from a sample containing many thousands of different proteins. Immuno-precipitation requires that the antibody be coupled to a solid substrate at some point in the procedure. There are 5 types of immuno-precipitation:

1. Individual protein Immuno-precipitation (IP), involves using an antibody that is specific for a known protein to isolate that protein out of a solution containing many different proteins. These solutions will often be in the form of a crude lysate of a plant or animal tissue. Other sample types could be bodily fluids or other samples of biological origin.

2. Protein complex immuno-precipitation (Co-IP), involves the immuno-precipitation of intact protein complexes (i.e. antigen along with any proteins or ligands that are bound to it) is known as co-immuno-precipitation (Co-IP). Co-IP works by selecting an antibody that targets a known protein that is believed to be a member of a larger complex of proteins. By targeting this known member with an antibody, it may become possible to pull the entire protein complex out of solution and thereby identify unknown members of the complex. This concept of pulling protein complexes out of solution is sometimes referred to as a "pull-down".

3. Chromatin immuno-precipitation (ChIP) is a method used to determine the location of DNA binding sites on the genome for a particular protein of interest. This technique gives a picture of the protein–DNA interactions that occur inside the nucleus of living cells or tissues. It is an in vivo method. DNA-binding proteins (including transcription factors and histones) in living cells can be cross-linked to the DNA that they are binding. By using an antibody that is specific to a putative DNA binding protein, one can immune-precipitate the protein–DNA complex out of cellular lysates.

4. RNA immuno-precipitation (RIP) targets RNA binding proteins. RIP is also an in vivo method in that live cells are lysed and the immuno-precipitation is performed with an antibody that targets the protein of interest. By isolating the protein, the RNA will also be isolated as it is bound to the protein.

5. Tagged proteins-tags engineered onto either the C- or N- terminal end of the protein of interest. The advantage here is that the same tag can be used time and again on many different proteins and the researcher can use the same antibody each time. Examples of tags in use are the Green Fluorescent Protein (GFP) tag, Glutathione-S transferase (GST) tag and the FLAG-tag.

Protein A Immuno-precipitation Kit (IP) can be used to specifically purify a protein from a complex mixture of proteins using a specific antibody and Protein A agarose beads that bind the antibody. The complex can be separated from the mixture by centrifugation. Subsequently, both antigen and antibody can be eluted from the Protein A beads.

Features :

- Fast and easy protocol - Immuno-precipitates a target protein in less than two hours is just 3 simple steps.
- Improves assay consistency.
- Efficient – Spin column provided eliminates resin loss and hence better recovery of target protein.

Kit Contents :

- Protease Inhibitor Cocktail (200X)
- Protein A Agarose
- Immuno precipitation Buffer
- 10% Sodium Deoxycholate
- 5% SDS
- Spin Columns

ReferencesFeatures :

- Harlow, E. and Lane, D. (1988). Antibodies: A Laboratory Manual. Cold Spring Harbor laboratory. Cold Spring Harbor, N. Y.
- Doolittle, M. H., et al., (1991) Analytical Biochemistry, 195, 364-368.

Ordering Information:

Cat. No	PI No.	Product Description
1660400011730	Kt144	Protein A Immunoprecipitation Kit, 20 preps

GeNei™ Quantitative Precipitin Assay Teaching Kit

Description :

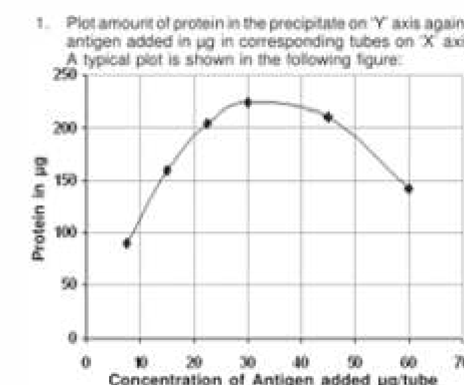
Polyclonal antibodies are obtained from immunized animals. These antibodies are used either directly or after processing to certain level of purity. In order to use these antibodies in immunological techniques it is essential to know the exact concentration of the active antibodies. One of the first observations of antigen-antibody interaction was their ability to precipitate when combined in certain proportions. Increasing amounts of antigen are added to a constant amount of antibody and the weight of precipitate formed in each tube is determined. The Quantitative Precipitin technique is a simple technique that is routinely used in the analysis of antibody and antigen interactions and for the estimation of the antibody as antigen content is a sample. It is based on the interaction of antibody and antigen to form a large protein complex that in certain solutions (buffer) will result in precipitation.

Using this kit, students will add a increasing amount of antigen to constant amount of antibody and determine the amount of antibody from the precipitate obtained. The difference in these two values is the amount of antibody present. Maximal precipitation occurs when all the antigen and all the antibody is incorporated into a lattice. When either antigen or antibody is in excess, smaller antigen-antibody complexes are former resulting in decreased precipitation.

GeNei™ DNA Molecular Size Determination Teaching Kit

Kit Contents :

- Antigen
- Antiserum
- 10X Assay Buffer
- 2X Resuspension Buffer
- 1.5 ml vials



- The quantitative precipitin assay enables environmental scientists to monitor and measure the presence of specific antigens or antibodies in environmental samples. This helps in identifying pollution sources, tracking pathogen outbreaks, and evaluating the impact of environmental factors on the immune system of organisms.
- The applications of the quantitative precipitin assay are diverse and encompass fields like medical diagnosis, immunological research, forensic science, food allergy testing, and environmental monitoring. Its ability to

References :

- Heidelberger, Michael; Kendall, Forrest E. (30 November 1929). "A Quantitative Study of the Precipitin Reaction Between Type III Pneumococcus Polysaccharide and Purified Homologous Antibody". Journal of Experimental Medicine.

Ordering Information:

Cat. No	PI No.	Product Description
6101100011730	KT11	GeNei™ Quantitative Precipitin Assay Teaching Kit, 10 expts.

GeNei™ Immuno-precipitation Teaching Kit

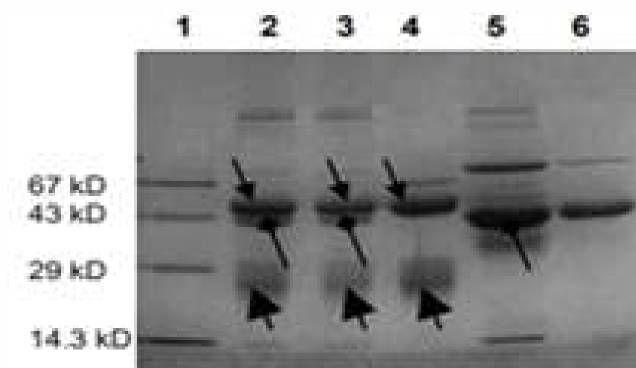
Description :

Immunoprecipitation (IP) is the technique of precipitating a protein antigen out of solution using an antibody that specifically binds to that protein. This process can be used to isolate and concentrate a particular protein from a sample containing many thousands of different proteins. Immunoprecipitation requires that the antibody be coupled to a solid substrate at some point in the procedure. In the Direct method, antibodies that are specific for a particular protein (or group of proteins) are immobilized on a solid-phase substrate such as microscopic agarose beads. The beads with bound antibodies are then added to the protein mixture and the proteins that are targeted by the antibodies are captured onto the agarose beads via the antibodies, in other words, they become immune-precipitated. In the In-direct method, antibodies that are specific for a particular protein, or a group of proteins, are added directly to the mixture of protein. The antibodies have not been attached to a solid-phase support yet. The antibodies are free to float around the protein mixture and bind their targets. As time passes, the beads coated in protein A/G are added to the mixture of antibody and protein. At this point, the antibodies, which are bound to their targets, will stick to the agarose beads. an be utilized in a number of downstream applications.

Using this kit, students will prepare a complex of Protein A Agarose (PAA) and antibody (from rabbit antiserum sample) is made which is further used for immuno-precipitation of Antigen (egg white in this case). The kit is also supplied with specific Antiserum (used to make a complex with PAA), Protein Standard (to help identify the different components of the PAA complex) and Antigen control (in this case ovalbumin), to compare with the crude egg white-PAA antibody complex.

Kit Contents :

- Protein A Agarose beads
- Antiserum
- Antigen (ovalbumin)
- Protein Standard
- Wash Buffer (10X)
- Acrylamide 30%
- Tris-SDS pH 8.8
- Tris-SDS pH 6.8
- Ammonium persulphate
- Reservoir Buffer (10X)
- Sample Loading Buffer
- Stainer 125 ml
- TEMED



Lane	Description
Lane 1	Protein Standard (Vial 3) with 4 prominent bands. with molecular weigh ranging from 67kD to 14.3 kD
Lane 2-3	Antigen-antibody-PAA immune-precipitated complex (vial 1)
Lane 4	vial 2 without antigen
Lane 5	Proteins present in Crude egg white (vial 5)
Lane 6	Antigen control (vial 4)

Note. →Indication antibody heavy chain
→Indication antibody light chain
→Indicates antigen

Analysis of Antigen-Antibody-PAA complex with appropriate control by SDS-PAGE

Ordering Information:

Cat. No	PI No.	Product Description
6112200011730	Kt122	GeNei™ Immuno-precipitation Teaching Kit, 5 expts.

Immunoelectrophoresis (IEP) Teaching Kit

Immunoelectrophoresis is a process for the identification of proteins in serum or other fluid separated into its component parts. The term "Immunoelectrophoresis" was first coined by Grabar and Williams in 1953.

It is a technique using a combination of protein electrophoresis and an antigen-antibody interaction, to separate mixtures of proteins and identify the electrophoresed antigens with antiserum to form precipitin bands. Antigens thus resolved by electrophoresis are subjected to immunodiffusion with antiserum added in a trough cut in the agarose gel. Due to diffusion, density gradient of antigen and antibody are formed and at zone of equivalence antigen antibody complex precipitates to form an opaque arc shaped line in the gel. The precipitin line indicates the presence of antibody, specific to the antigen. If the antibody is homogeneous only one precipitin line is visible. Presence of more than one precipitin line establishes the heterogeneity of antibody, while the absence of precipitin line indicates that the antiserum does not have antibody to any of the antigens separated by electrophoresis.

In this kit, 2 types of antisera raised against a particular antigen are supplied. Antiserum A supplied has antibodies against rabbit whole serum raised in goat. It thus contains a mixture of antibodies against serum proteins and will thus form more than one precipitin line indicating the heterogeneity of the antisera. While Antiserum B supplied has antibody against rabbit IgG raised in goat. Since it contains a single antibody, only one precipitin line will be formed indicating the specificity of the antibody against the antigen. Following Immunoelectrophoresis, students will be able to establish the homogeneity/heterogeneity of the antisera.

Available Picture

Slide showing precipitin lines following immunoelectrophoresis.

Kit Contents :

- Agarose
- 5X Electrophoresis buffer
- Antigen
- Test Antiserum-A
- Test Antiserum-B

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Ordering Information:

Cat. No	PI No.	Product Description
6102000011730	KT20A	GeNei™ Immunoelectrophoresis Teaching Kit, 5 expts.
6102000021730	KT20B	GeNei™ Immunoelectrophoresis Teaching Kit, 10 expts.
6102000031730	Kt20	GeNei™ Immunoelectrophoresis Teaching Kit with ETS2, 5 expts.

GeNei™ Latex Agglutination Teaching Kit

Description :

Latex agglutination is observed when a sample containing the specific antigen (or antibody) is mixed with an antibody (or antigen) which is coated on the surface of latex particles. All methods of detecting or quantitating antigen or antibody take advantage of the fact that they react to form a complex. At the optimum antigen-antibody concentration, this complex precipitates out. However, if the antigen is particulate in nature, agglutination of antigen-antibody complex is observed.

Agglutination Reactions: The reaction between a particulate antigen and an antibody result in visible clumping called agglutination. Antibodies that produce such reactions are known as agglutinins. The principle of Agglutination reactions is similar to precipitation reactions; they depend on the cross linking of polyvalent antigens. When the antigen is an erythrocyte, it is called hemagglutination. Theoretically all antibodies can agglutinate particulate antigens but IgM, due to its high specificity is a particularly good agglutinin. There is no agglutination observed when the concentration of antibody is high, (lower dilutions), and then the sample is diluted, agglutination occurs. Prozone effect is defined as the invisibility of agglutination at high concentrations of antibodies. It is due to the reason that excess antibody forms very minute complexes that do not clump to form visible agglutination. The latex agglutination test is a clinical method to detect certain antigens or antibodies in a variety of bodily fluids such as blood, saliva, urine or cerebrospinal fluid.

The sample Latex agglutination test includes some of the advantages. They are: 1. Ability to obtain semi quantitative results. 2. A low individual test cost. 3. Relatively short time to obtain results to be tested is sent to the lab and where it mixed with latex beads coated with a specific antigen or antibody. The clumping of latex beads (agglutination) indicates the presence of suspected particles. By performing 2- to 10-fold dilutions of specimens we can obtain the semi quantitative results. Latex agglutination test has some disadvantages also which include 2. Need to carefully interpret marginal results and 3. Problems with specificity due to interfering substances in many assays. Positive result will show development of an agglutinated pattern showing clearly visible clumping of the latex particles. Negative result will show no agglutination and the milky appearance remains unchanged throughout the test. Latex agglutination tests have been applied in clinical laboratories for the detection of infectious diseases and in 1956 Singer and Plotz first described Rheumatoid Factor Test, a test based on latex agglutination. In rheumatoid arthritis (RA), IgG antibodies produced by lymphocytes in the synovial joint react with the IgM antibodies (RF, rheumatoid factor) to generate immune complexes that activate the complement and cause the tissue destruction. The RA is of diagnostic significance. Since then, tests to detect microbial and viral infections, autoimmune diseases, hormones, drugs and serum proteins have been developed and marketed by many companies worldwide. The principle is used for diagnosing many infections such as Hepatitis B, H. influenzae, N. meningitis, etc

In this kit, Latex Beads, Antigen and Test Antiserum are provided. The experiment involves coating of the latex beads with the Antigen followed by blocking of the unreacted sites. The coated latex beads are then reacted with the Test Antiserum and if the Antiserum is specific for the antigen coated on them agglutination is observed. The specificity of the agglutination is further checked by Agglutination Inhibition Reaction.

Kit Contents :

- Latex Beads
- Glycine-Saline Buffer
- Blocking Buffer
- Antigen for Coating
- Test Antiserum
- Glass Plates

Ordering Information:

Cat. No	PI No.	Product Description
6105300011730	Kt53	GeNei™ Latex Agglutination Teaching Kit, 10 expts.
6105300021730	KT53A	GeNei™ Latex Agglutination Teaching Kit, 20 expts.

GeNei™ Counter Current Immunoelectrophoresis Teaching Kit

Description :

CCIEP is a rapid version of Ouchterlony Double Diffusion (ODD) technique and can be performed within an hour. It is primarily a qualitative test, although from the thickness of the precipitin line relative measure of quantity can be obtained. The antigen is placed in a well at the cathode and antibody is placed at the anode. During electrophoresis molecules placed in an electric field acquire a charge depending on their pI (Isoelectric Point). Hence they move towards the appropriate electrode. For e.g. negatively charged antigen moves towards the anode. The Antibody (Immunoglobulin) at pH 7.6 has a charge nearing zero. During electrophoresis, the agarose matrix absorbs OH- ions on the surface resulting in a net increase in positive ions at a distance from the matrix. These positive ions migrate towards the negative pole with a solvent shield, resulting in a net solvent flow called Endosmosis. Hence antibody molecules which have no charge move towards cathode along with solvent shield due to this phenomenon. Thus the antigen and antibody travel towards each other and at a point where there is optimum concentration of both a Line Of Precipitation (band) is formed.

Using this kit, students will test for the presence of antibody in 3 different test antisera samples. The antigen supplied is Bovine Serum Albumin (BSA), which has negative charge at pH 7.6. BSA will be loaded into wells near the Cathode and antisera (positive control and 3 test samples) towards the Anode. During electrophoresis, the antigen will migrate towards the anode and the antibody towards the cathode. At the equivalence point, a precipitin line will be formed in those antisera samples which have antibody against BSA.

Kit Contents :

- Antigen
- Test Antiserum
- Positive Control
- Antiserum Electrophoresis Buffer (5X)
- Agarose

Cat. No	PI No.	Product Description
6102900011730	KT29A	**GeNei™** Counter Current Immunoelectrophoresis Teaching Kit,10 expts

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Dot ELISA Teaching Kit

Description :

Dot-ELISA is a special modification for ELISA assay. The Dot-ELISA, short form for the dot enzyme-linked immunosorbent assay, stands as a remarkably versatile solid-phase immunoassay designed for the detection of antibodies or antigens. The application of minuscule quantities of reagents, which are meticulously dotted onto solid surfaces, including materials like nitrocellulose and other paper membranes that possess a strong affinity for binding proteins. After incubation with antigen-specific antibody and enzyme-conjugated anti-antibody, the addition of a precipitable, chromogenic substrate causes the formation of a coloured dot on the solid phase which is visually read.

It has more advantages than the indirect ELISA as it can be performed using a minute volume of reagents and is easily read visually.

This kit equips students with the knowledge and hands on skills required for Dot-ELISA and for the analysis against the standard

Kit Contents :

- Dot ELISA Strip
- 10X Assay Buffer
- Antibody-HRP Conjugate
- 10X TMB/H2O2
- Test Serum Samples (A, B & C)

References :

- Recent applications of the Dot-ELISA in immunoparasitology author links open overlay panel Michael G. Pappas*

Ordering Information:

Cat. No	PI No.	Product Description
6101200011730	KT12S	GeNei™ DOT ELISA Teaching Kit, 15 expts

GeNei™ Antigen Capture ELISA Teaching Kit, (Competitive ELISA)

Description :

ELISA or enzyme linked immunosorbent assay is a sensitive laboratory method used to detect the presence of antigens (Ag) or antibodies (Ab) of interest in a wide variety of biological samples. These assays require an immunosorbent i.e., antigen or antibody immobilized on solid surface such as wells of microtiter plates or membranes. Antigen capture ELISA is the most useful immunosorbent assay for detecting antigen, since it is 2-5 fold more sensitive than assays in which antigen is directly bound onto the solid phase. In this assay, constant and limiting amount of antibody is immobilized onto a solid support. A fixed amount of labelled antigen [i.e., antigen coupled with enzyme like Horse Radish Peroxidase (HRP), alkaline phosphatase (ALP) etc.] is added and allowed to compete with unlabelled antigen (Standard or Test Sample) for the immobilized antibody. The amount of labelled antigen bound is then estimated by a suitable assay for the label. The amount of labelled antigen that binds is inversely proportional to the amount of unlabelled antigen in the reaction mixture. Thus, the estimate of label in the well decreases with increase in the antigen concentration in the Standard or Test Sample.

In this kit, an Antibody for immobilization, unlabelled antigen (Standard and Test) and HRP Labelled Antigen are supplied. Students will immobilize the Antibody in the wells of a microtiter plate. To this they will add unlabelled antigen (Test Antigen or varying amounts of Standard Antigen) and a fixed amount of HRP Labelled Antigen. The two antigens compete to bind to immobilized Antibody.

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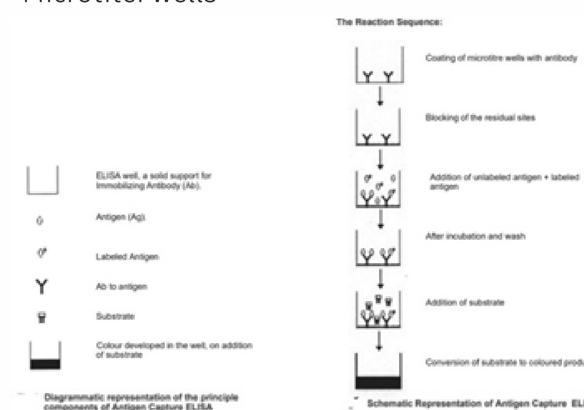
At higher concentrations of Standard Antigen, the amount of Labelled Antigen that binds to the antibody will be lower. The amount of HRP-Labelled Antigen bound will then be estimated using Hydrogen Peroxide (H2O2) as the substrate and Tetramethylbenzidine (TMB) as a chromogen. HRP acts on H2O2 to release nascent oxygen, which oxidizes TMB to TMB oxide, a blue-coloured product. The intensity of the colour will be measured spectrophotometrically. Hence, by using various amounts of the Standard Antigen, students will plot a standard curve and then determine the concentration of the antigen in the three Test Samples provided.

Kit Contents :

- Standard Antigen
- 100X Antibody
- Test Samples (1, 2 and 3)
- 1000X HRP Labelled Antigen
- Blocking Buffer
- 10X TMB/H2O2
- Coating Buffer
- 10X PBST
- 5X Stop Solution
- Microtiter wells



Diagrammatic representation of the principle components of Antigen Capture ELISA

Schematic Representation of Antigen Capture ELISA

References :

- Engvall, E (1972-11-22). "Enzyme-linked immunosorbent assay, Elisa". The Journal of Immunology. 109 (1): 129–135.
- Crowther, J.R. (1995). "Chapter 2: Basic Principles of ELISA". ELISA: Theory and Practice. Methods in Molecular Biology.

Ordering Information:

Cat. No	PI No.	Product Description
6105000011730	KT50	GeNei™ Antigen Capture ELISA Teaching Kit, (Competitive ELISA) 4 expts.

GeNei™ Antibody Capture ELISA Teaching Kit, (Indirect ELISA)

Description :

ELISA or enzyme linked immunosorbent assay is a sensitive laboratory method used to detect the presence of antigens (Ag) or antibodies (Ab) of interest in a wide variety of biological samples. These assays require an immunosorbent i.e., antigen or antibody immobilized on solid surface such as wells of microtitre plates or membranes. Indirect ELISA or antibody capture ELISA method is useful for screening an antiserum for specific antibodies. Antibodies are detected by coating the wells of microtitre plate with the antigen and incubating the coated wells with test solution containing specific antibodies (primary antibody). Unbound antibodies are washed and the bound antibody is detected with a secondary antibody conjugated to an enzyme [eg., Horse radish peroxidase (HRP), alkaline phosphatase (ALP) etc.]. After incubation, unbound conjugate is washed and substrate solution is added. After a second incubation, amount of substrate converted to coloured product is assessed using a spectrophotometer. The measured amount is directly proportional to the amount of antibody or the antigen in the test solution.

In this kit, a Standard Primary Antibody and three test Antibody Samples are supplied. Students will immobilize the corresponding antigen in the wells and to this add primary antibody (test antibody or varying concentrations of the Standard primary antibody). The amount of primary antibody bound

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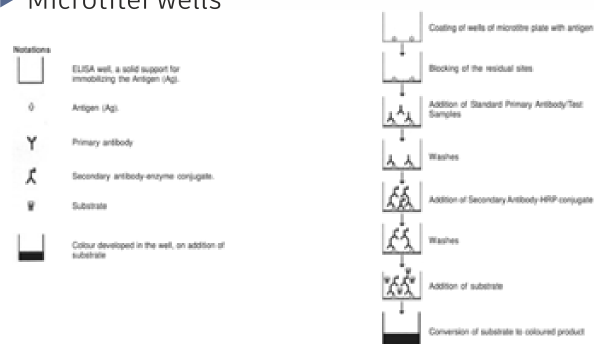
will be detected with a Secondary Antibody conjugated to Horse Radish Peroxidase (HRP), which in turn is detected by incubating with substrate, Tetramethylbenzidine (TMB) requiring H_2O_2. HRP acts on the H_2O_2 to release nascent oxygen, which in turn acts on the chromogen, Tetramethylbenzidine (TMB) resulting in the formation of a blue coloured product. The intensity of the colour is directly proportional to the amount of secondary antibody conjugate bound to the primary antibody and is measured spectrophotometrically. Hence, by using various amounts of Standard Primary Antibody, students will plot a Standard Curve and then determine the concentration of the antibody in the three Test Samples provided.

Kit Contents :

- Antigen for coating
- Standard Primary Antibody
- Test Samples (1, 2 and 3)
- 1000X Secondary antibody-HRP conjugate
- Blocking Buffer
- 10X TMB/H_2O_2
- Coating Buffer
- 10X PBST
- 5X Stop Solution
- Microtiter wells



References :

- Engvall, E (1972-11-22). "Enzyme-linked immunosorbent assay, Elisa". The Journal of Immunology. 109 (1): 129–135.
- Crowther, J.R. (1995). "Chapter 2: Basic Principles of ELISA". ELISA: Theory and Practice. Methods in Molecular Biology.

Ordering Information:

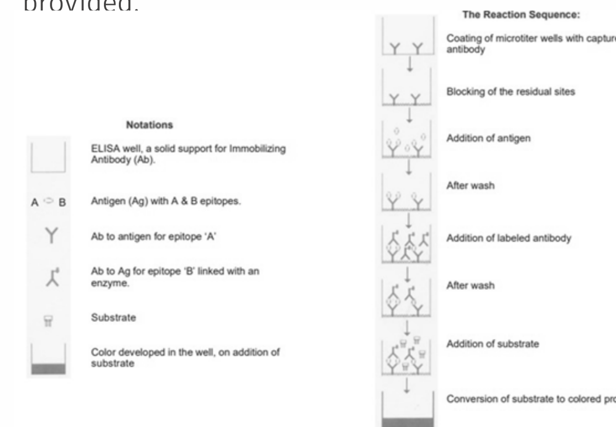
Cat. No	PI No.	Product Description
6105100011730	KT51	**GeNei™** Antibody Capture ELISA Teaching Kit, (Indirect ELISA) 4 expts.

GeNei™ Sandwich ELISA Teaching Kit

Description :

ELISA or Enzyme Linked Immunosorbent Assay is a sensitive laboratory method used to detect the presence of antigens (Ag) or antibodies (Ab) of interest in a wide variety of biological samples. This assay requires an immunosorbent, i.e., antigen or antibody immobilized on solid surface such as the wells of microtiter plates or membranes. In this method, two antibodies that can bind to two different epitopes on the same antigen are required. One of the antibodies is immobilized on a microtiter well and is referred to as capture antibody and the other antibody is labelled with a suitable enzyme [e.g., horse radish peroxidase (HRP), alkaline phosphatase (ALP) etc.] and is referred to as labelled antibody. Sample (standard and test) containing the antigen is allowed to react with the immobilized antibody. After the well is washed, labelled antibody is added and allowed to react with the bound antigen. Unreacted labelled antibody is washed out and the enzyme bound to solid support is estimated by adding a chromogenic substrate. The colour developed is measured spectrophotometrically which is directly proportional to the antigen concentration.

In this kit, a Standard Antigen, three Test Samples, Capture Antibody and Antibody-HRP Conjugate are supplied. Students will immobilize the Capture Antibody in the ELISA wells and to this add antigen (Test Antigen or varying amounts of Standard Antigen). The amount of antigen bound will be detected by the addition of HRP labelled antibody, which in turn will be estimated using Tetramethylbenzidine (TMB) as substrate. HRP acts on H_2O_2 to release nascent oxygen which oxidizes TMB to TMB oxide, a blue-coloured product. The intensity of the colour will be measured using a spectrophotometer at 450 nm. Hence, by using various amounts of the Standard Antigen, students will plot a standard curve and then determine the concentration of antigen in three Test Samples provided.



Kit Contents :

- Antigen for coating
- Standard Primary Antibody
- Test Samples (1, 2 and 3)
- 1000X Secondary antibody-HRP conjugate
- Blocking Buffer
- 10X TMB/H_2O_2
- Coating Buffer
- 10X PBST
- 5X Stop Solution
- Microtiter wells

Kit Contents :

- Standard Antigen
- 100X Capture Antibody
- Test Samples (1, 2 and 3)
- 1000X Antibody-HRP Conjugate
- Blocking Buffer
- 10X TMB/H_2O_2
- Coating Buffer
- 10X PBST
- 5X Stop Solution
- Microtiter Wells

References :

- Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides. 2015 Oct;72:4-15.
- Engvall E. The ELISA, enzyme-linked immunosorbent assay. Clin Chem. 2010 Feb;56(2):319-20.
- Shah K, Maghsoudlou P. Enzyme-linked immunosorbent assay (ELISA): the basics. Br J Hosp Med (Lond). 2016 Jul;77(7):C98-101.
- Konstantinou GN. Enzyme-Linked Immunosorbent Assay (ELISA). Methods Mol Biol. 2017;1592:79-94.

Ordering Information:

Cat. No	PI No.	Product Description
6105200011730	KT52	**GeNei™** Sandwich ELISA Teaching Kit, 4 expts

Rocket Immuno-electrophoresis (RIEP))

Description :

Rocket Immunoelectrophoresis (also referred to as electro immunoassay) is a simple, quick, and reproducible method for determining the concentration of a specific protein in a protein mixture. The method, originally introduced by Laurell involves a comparison of the sample of unknown concentration with a series of dilutions of a known concentration of the protein and requires a monospecific antiserum against the protein under investigation.

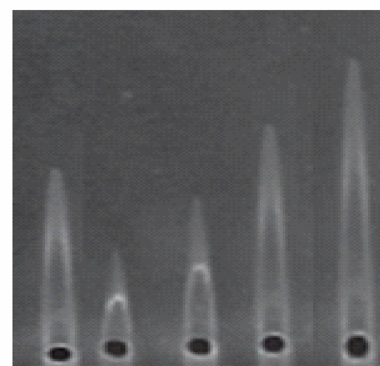
As the samples electrophorese farther through the gel, more antibody molecules are encountered that interact with the antigen and when the "equivalence point" is reached, the Ag-Ab complex precipitates. This precipitin line is seen in the form of of the precipitin bands in the shape of cone-like structures (rocket appearance) at the end of the reaction.

This kit equips students with the knowledge and hands on skills required for RIEP preparation and helps with the analysis of the precipitin bands formed.

Kit Contents :

- 5X Electrophoresis Buffer
- Antiserum*
- Standard Antigen (A, B, C & D) (each)
- Test Antigen (1 and 2)

Ordering Information:



Sample	Std conc(mg/mL)	Rocket height(cm)
Test	-	19
A	0-125	11
B	0.25	18
C	0.5	27
D	1.0	39

Standard concentration with series of rockets of increasing heights(A,B,C,D) are seen that is proportional to amount of antigen in the well. Therefore, a direct measurement of the height of rocket will reflect upon the antigen concentration which will determine the concentration of the test. A standard graph of antigen concentration versus peak height is then constructed and from the peak height of the unknownsample, concentration of antigen is determined.

References :

https://microbenotes.com/rocket-immunoelectrophoresis/

Ordering Information:

Cat. No	PI No.	Product Description
6104700011730	KT47A	GeNei™ Rocket Immunoelectrophoresis Teaching Kit, 5 expts.

Ouchterlony Double Diffusion (ODD) Teaching Kit

(For Antibody Titration)

Description :

The Ouchterlony Double Diffusion (ODD) test centers on the formation of a distinct antigen-antibody precipitin line within an agar gel medium, making it an invaluable tool for the screening of both antigens and antibodies.

In the figure below, we observe a typical antibody titration using the kit. The antibody dilutions, starting with 1;2 upto 1:32, are strategically placed in the wells in a clockwise sequence beginning with Ab-1. What becomes evident is that the intensity of the precipitin line decreases as the antibody dilution increases. This decline in intensity is a crucial observation in the antibody titration process.

Antibodies have at least two antigen binding sites, thus large aggregates or lattices of antigen and antibody are formed. Precipitation will not occur if excess antigen is present or if excess antibody is present. Cross- linking and lattice formation will only occur when antigen and antibody concentrations are optimal. An increasing amount of antigen is added to a constant amount of antibody in solution. This is called the antibody-excess zone (Prozone phenomenon). The Ag and Ab concentrations are relatively higher near their respective wells. As they diffuse farther from the wells, their concentration decreases. An antigen will react with its specific antibody to form an Ag-Ab complex. As more antigens are added, the amount of protein precipitated increases until the antigen/antibody molecules are at an optimal ratio. This is known as the equivalence zone or equivalence point. When the amount of antigen in solution exceeds the amount of antibody, the amount of precipitation will decrease. This is known as the antigen excess zone (Prozone phenomenon).

This kit equips students with the knowledge and hands on skills required for ODD preparation and helps with the analysis of the antibody titration.

Kit Contents :

- Agarose
- 10X Assay Buffer
- Antigen
- Test Antiserum
- Glass Plate
- Gel Punch with Syringe
- Template

Antisera dilutions in wells 1-6:

Well No.	Dilution of Test Antiserum
1.	Neat
2	1:2
3	1:4
4	1:8
5	1:16
6	1:32

The centre well contains antigen.

Titre value: A, 1:2 B, 1:16

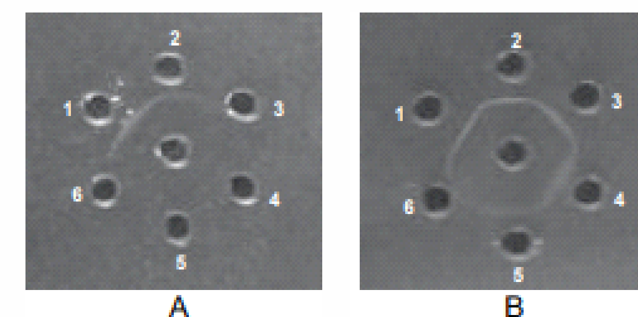


Figure :

Typical ODD pattern for antibody titration.

References :

https://microbenotes.com/ouchterlony-double-immunodiffusion-technique/

Ordering Information:

Cat. No	PI No.	Product Description
6100900011730	KT09S	GeNei™ Ouchterlony Double Diffusion Teaching Kit for Antibody Titration, 15 expts.

Radial Immunodiffusion (RID) Teaching Kit

Description :

Single Radial Immunodiffusion, also known as Mancini technique, is a quantitative immunodiffusion technique used to detect the concentration of antigen by measuring the diameter of the precipitin ring formed by the interaction of the antigen and the antibody at optimal concentration.

The antigen-antibody precipitation is made more sensitive by the incorporation of antiserum in the agarose. Antigen (Ag) is then allowed to diffuse from wells cut in the gel in which the antiserum is uniformly distributed. Initially, as the antigen diffuses out of the well, its concentration is relatively high and soluble antigen-antibody adducts are formed. However, as Ag diffuses farther from the well, the Ag-Ab complex reacts with a larger amount of antibody resulting in a lattice that precipitates to form a precipitin ring.

The size of the precipitin rings depends on:

- Antigen concentration in the sample well
- Antibody concentration in the agarose gel
- Size of the sample well
- Volume of the sample.

This kit equips students with the knowledge and hands on skills required for RID preparation and helps with the analysis of the precipitin ring formed.

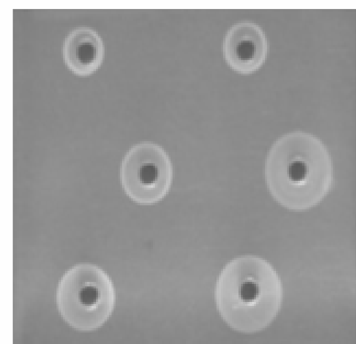
GENOMICS

Molecular cloning or gene cloning involves insertion of a DNA segment of interest into an autonomously replicating DNA molecule, i.e., a cloning vector. Transforming the vector into a suitable host organism results in the production of large amounts of the inserted DNA fragment. For expression of genes, the insert DNA should be flanked by correctly oriented control sequences for RNA and protein synthesis. Hence, one uses an expression vector, such that the host produces large quantities of RNA

Kit Contents :

- Agarose
- 10X Assay Buffer
- Standard Antigens (A, B, C & D)
- Test Antigen (1 & 2)
- Antiserum
- Gel Punch with syringe
- Glass Plate
- Template

RID test results using the kit is shown in the below figur



Sample No.	Std. Ag Conc. (in mg/ml.)	Ring Diameter (in mm)
A	0.25	6
B	0.5	8
C	1.0	10
D	2.0	12
Test Antigen 1	1.5	11
Test Antigen 2	0.7	9

By loading a range of known antigen concentrations on the gel and by measuring the diameters of their precipitin rings, a calibration graph is plotted. Concentrations of unknown antigens, can be determined by measuring the diameter of precipitin rings and extrapolating this value on the calibration graph

References :

Immunological Methods in Microbiology Sukhadeo B. Barbuddhe, ... Deepak B. Rawool, in Methods in Microbiology, 2020

Ordering Information:

Cat. No	PI No.	Product Description
6101000011730	KT10S	GeNei™ Radial Immuno Diffusion Teaching Kit, 15 expts.

Ouchterlony Double Diffusion (ODD) Teaching Kit

(For Antigen Antibody Patterns)

Description :

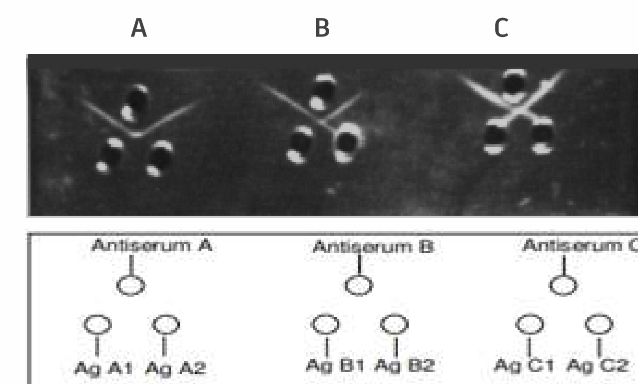
The Ouchterlony double diffusion (ODD) method stands as one of the fundamental techniques for testing antisera to detect antibodies against a specific antigen and to determine their quantity. This ingenious method owes its existence to the brilliant mind of Orjan Ouchterlony.

This approach has found extensive application in the realm of detection and qualitative diagnostic procedures. What makes it truly unique is the "double" aspect. In this process, both antibodies and antigens are allowed to migrate towards each other within a gel medium, and where they meet, a specific precipitation line materializes. This precipitation phenomenon is highly selective and discriminative.

This kit equips students with the knowledge and hands on skills required for ODD preparation and helps with the analysis of the similarity between the antigens.

Kit Contents :

- Agarose
- 10X Assay Buffer
- Antiserum (A, B and C)
- Test Antigens (A1, A2, B1, B2, C1, C2) (each)
- Glass Plate
- Gel Punch with Syringe
- Template



GST Protein analyzed on SDS-PAGE before and after IPTG induction.

Figure :

Plate showing pattern of lines obtained following Ouchterlony double diffusion.

References :

https://microbenotes.com/ouchterlony-double-immunodiffusion-technique/

Ordering Information:

Cat. No	PI No.	Product Description
6107000011730	Kt70	**GeNei™** Ouchterlony Double Diffusion Teaching Kit for Antigen-Antibody patterns, 15

GeNei™ Western Blotting Teaching Kit

Description :

Immunoblotting or western blotting technique was introduced by Towbin, et al. in1979 its precise identification of target proteins within a complex mixture of unrelated protein species makes it widely used for protein analysis. The specificity of the antibody-antigen interaction enables a target protein to be identified amid a complex protein mixture. Western blotting can produce qualitative and semi-quantitative data about the protein of interest.

The process typically unfolds as follows:

- Proteins are first separated through electrophoresis and then transferred onto membranes, typically nitrocellulose membranes or PVDF followed by blocking.
- The membrane is then treated with a primary antibody to recognize the specific target protein.
- Subsequently, a secondary antibody is applied, which is often labeled with enzymes, for visual detection chromogenic substrate is used.

This kit equips students with the knowledge and hands on skills required for western blotting preparation and analysis of the blot.

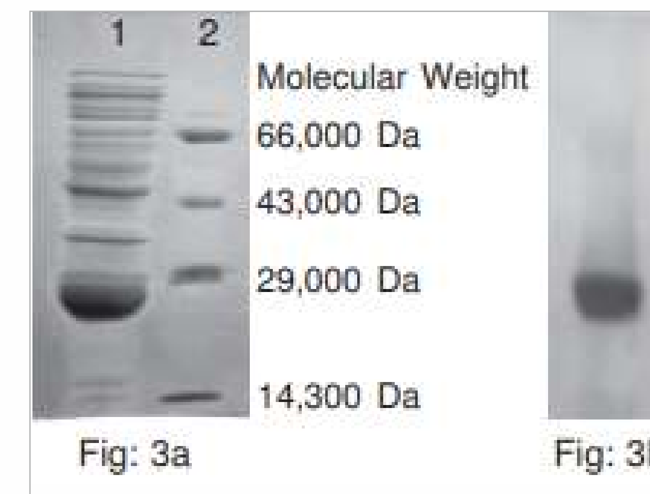


Fig: 3a Fig: 3b

Lane 1 - Bacterial Lysate

Lane 2 - Protein Marker - Standard proteins ranging in molecular weight from 66 kD - 14.3 kD

Kit Contents :

- Acrylamide 30%
- Tris-SDS pH 8.8
- Tris-SDS pH 6.8
- Sample Loading Buffer
- Protein Standard
- Protein sample*
- 10X Diluent Buffer
- 10X Assay Buffer
- 25X Wash Buffer
- Primary Antibody*
- 1000X HRP Conjugate
- 10X TMB/H2O2
- TEMED
- Ammonium persulphate (APS)
- 10X Reservoir Buffer
- 20X Blotting Buffer Component A
- 20X Blotting Buffer Component B
- Blocking Agent
- Nitrocellulose (NC)
- Filter Paper
- Stainer

Figure :

Protein bands as detected by Coomassie blue staining of SDS-Polyacrylamide gel and Immunodetection of GST on blotted NC membrane.

References :

https://microbenotes.com/western%20blotting/

Ordering Information:

Cat. No	PI No.	Product Description
6102100011730	KT21A	GeNei™ Western Blotting Teaching Kit, 5 expts.
6102100021730	KT21B	GeNei™ Western Blotting Teaching Kit, 20 expts.
6102100031730	Kt21	GeNei™ Western Blotting Teaching Kit with ETS3, 5 expts.

GeNei™ Antibody-HRP Conjugation Teaching Kit

Description :

The most commonly used method for labeling IgG molecules with Horse Radish Peroxidase (HRP) exploits the glycoprotein nature of the enzyme. The saccharide residues of HRP are oxidized with Sodium Meta-periodate to produce aldehyde groups that can react with the amino groups of the IgG molecule and the Schiff base formed is then reduced by Borohydride to give a stable conjugate. Desalting is done using a gel filtration column to remove the Borohydride. The conjugate is stored in PBS. The efficacy of the enzyme-labeled antibody is tested by direct Dot-ELISA and is expressed as the titre value of the conjugate. Nitrocellulose membrane on which the appropriate antigen is immobilized, is incubated with various dilutions of the conjugate. The amount of HRP-labeled antibody bound is assayed by adding the substrate for HRP, Tetramethylbenzidine (TMB/H2O2). HRP acts on the hydrogen peroxide to release nascent oxygen that oxidizes TMB, a chromogen giving a blue coloured product, TMB oxide.

In this kit, the antibody supplied will be labeled with HRP by the periodate method. Following labeling and desalting, the titre of the antibody-HRP conjugate will be determined by direct Dot-ELISA, using antigen spotted and blocked Nitrocelluose strips, provided with the kit.

Kit Contents :

- Antigen Spotted Strip
- Antibody for Coupling
- Carbonate Buffer
- Desalting Column
- Oxidation Tubes
- Reductant Solution
- Stabilizer
- 10X ELISA Buffer
- 10X PBS (Phosphate Buffered Saline)
- 10X TMB/H2O2

References :

- Engvall E. The ELISA, enzyme-linked immunosorbent assay. Clin Chem. 2010 Feb;56(2):319-20.
- Shah K, Maghsoudlou P. Enzyme-linked immunosorbent assay (ELISA): the basics. Br J Hosp Med (Lond). 2016 Jul;77(7):C98-101.

Ordering Information:

Cat. No	PI No.	Product Description
6104800011730	KT48	**GeNei™** Antibody-HRP Conjugation Teaching Kit, 5 expts.

GeNei™ Gel Filtration Chromatography Teaching Kit

Description :

Gel-filtration chromatography, also called size-exclusion or gel-permeation chromatography, separates molecules based on the differences in their size. The sample is applied on top of a column containing porous beads. As the molecules pass through the column of porous beads of crosslinked agarose, they get separated as follows:

- Large molecules cannot enter the pores and elute as the first peak in the chromatogram. They elute fast and this is called total exclusion.
- Intermediate molecules may enter the pores and may have an average residence time in the particles depending on their size and shape. Different molecules therefore have different total transit times through the column. This portion of a chromatogram is called the selective permeation region.

·Small molecules enter the pores and have the longest residence time in the column and elute together as the last peak in the chromatogram. This last peak in the chromatogram is the total permeation limit.

In this kit, a mixture of three different biomolecules ranging in molecular size from 376 Da to 2000 kDa are supplied. These are separated based on their size through a gel filtration column. The movement of the samples through the column is easily monitored as the biomolecules are coloured, which also aids in collection of the resolved biomolecules.

Kit contents :

- Gel Filtration Column
- Gel Filtration Buffer
- Sample

References :

- Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology (8 eds.). Cambridge University Press: New York.
- www.ncbi.nlm.nih.gov/pmc/articles/PMC5206469/

Ordering Information:

Cat. No	PI No.	Product Description
6103900011730	KT39	**GeNei™** Gel Filtration Chromatography Teaching Kit, 5 expts.

GeNei™ Ion Exchange Chromatography Teaching Kit

Description :

Ion exchange chromatography works on the basic principle that oppositely charged particles are attracted to each other. The stationary phase consists of fixed charges on a solid support. These charges can be either negative or positive. Hence, there are two types of ion exchangers i.e., cation and anion exchangers. Cation exchangers possess negatively charged groups and attract positively charged molecules, eg, Carboxymethylcellulose or CM-cellulose. Conversely, anion exchangers have

positively charged groups that attract negatively charged molecules and thus separate anionic molecules. eg, Diethylaminoethyl-cellulose (DEAE Cellulose). Proteins are complex ampholytes i.e., they have both positive and negative charges and can be separated from a mixture of compounds on the basis of net positive or negative charge that they carry. The Isoelectric point of a protein (pI) is the pH at which its net charge is zero (i.e., number of positive and negative charges are equal). Therefore, proteins will have either a net negative charge or net positive charge depending on the pH of the solution and thus, it is possible to use either an anion exchanger or a cation exchanger for purification

In ion exchange chromatography, a solution containing protein of interest is applied to the ion exchanger. The binding of protein to the ion exchanger is dependent on net charge of the protein at that particular pH and on the ionic strength of the mobile phase. The Bound protein is then eluted out from the stationary phase by increasing the concentration of counter ions or by changing the pH, which alters the charge on the protein. A weakly charged protein is displaced from the stationary phase with lower concentration of counter ions than a highly charged protein. This results in separation of protein based upon its net charge. The extent of purification of a protein (eg., enzyme) can be determined by computing its specific activity. Specific activity is the ratio of enzyme activity to mass of protein in the sample, usually expressed as units of activity per milligram of protein (U/mg). As the enzyme is purified (through a number of steps) other proteins in the mixture are eliminated while most of the enzyme activity is retained. This results in an increase in the specific activity of the enzyme. Hence, by determining the specific activity before and after purification, one can determine the fold purification and yield of the enzyme.

Using this kit, students will carry out purification of lysozyme from chicken egg white by Ion Exchange Chromatography. The pI of lysozyme is 10.5 and it carries a net positive charge at pH below 10.5. Hence, at pH 7.0 it binds to negatively charged column or a cation exchanger, CM-cellulose supplied in the kit. The Wash buffer (pH 9.0) will then be used to remove the proteins that have pI less than or equal to 9.0. The lysozyme will be eluted out by increasing concentration of cations. Cations compete with the positively charged groups of lysozyme for binding sites on the column, resulting in the elution of lysozyme

Kit Contents :

- CM-Cellulose
- 10X Equilibration Buffer (pH7.0)
- 10X Wash Buffer (pH 9.0)
- 5X Elution Buffer
- Neutralizing Solution
- 5M Sodium chloride
- 0.5 M Phosphate Buffer (pH 7.0)
- Tube for mixing
- Micrococcus luteus
- Lysozyme Standard
- Column

References :

- Okada T. Nonaqueous ion-exchange chromatography and electrophoresis Approaches to nonaqueous solution chemistry and design of novel separation. Journal of Chromatography A 1998; 804 17-28.
- Levison PR. Large-scale ion exchange column chromatography of proteins Comparison of different formats. Journal of Chromatography B 2003;790 17-33.

Ordering Information:

Cat. No	PI No.	Product Description
6104000011730	KT40	**GeNei™** Ion Exchange Chromatography Teaching Kit, 5 expts.

GeNei™ Affinity Chromatography Teaching Kit

Description :

Affinity chromatography is a method of selectively and reversibly binding proteins to a solid support matrix based on the fact that biological affinities exist between molecules, e.g., antigen with antibody. One of the components, the ligand is immobilized on a solid matrix, which is then used to selectively purify the target protein. By Including a competing ligand in the mobile phase or by changing the pH, the target protein is eluted. The performance of affinity chromatography is determined by comparing the specific activity of the protein before and after affinity purification. The Specific activity of the protein (enzyme) is defined as its activity per mg of protein. In order to determine the specific activity, enzyme activity and protein concentration are determined. Lectins are plant proteins that have high affinity for particular sugars such as those present in glycoproteins. HRP is a glycoprotein, which helps in its selective binding to the column. Pure protein will then be eluted out using a free sugar (eg., fructose) that competes with the glycoproteins for the immobilized lectin. HRP acts on hydrogen peroxide to release nascent oxygen that oxidizes ABTS to give coloured product.

Using this kit, students will carry out affinity purification of Horse Radish Peroxidase (HRP) from crude extract using a lectin column (Con A).

Kit Contents :

- ABTS
- Column
- Concanavalin A- Agarose suspension
- Suspension Crude Sample
- Elution Buffer
- Hydrogen Peroxide
- Sodium Acetate Buffer
- BSA (Protein Standard)
- Solution I
- Solution II
- Solution III

References :

- Zachariou, Michael, ed. (2008). Affinity Chromatography: Methods and Protocols (2nd ed.). Totowa, N.J.: Humana Press. pp. 1–2.
- Singh, Naveen K.; DSouza, Roy N.; Bibi, Noor S.; Fernández-Lahore, Marcelo (2015). "Direct Capture of His6-Tagged Proteins Using Megaporous Cryogels Developed for Metal-Ion Affinity Chromatography

Ordering Information:

Cat. No	PI No.	Product Description
6104100011730	Kt41	**GeNei™** Affinity Chromatography Teaching Kit, 5 expts.

GeNei™ Immunoglobulin G Isolation Teaching Kit

Affinity chromatography is a method of selectively binding protein to its corresponding affinity ligand, immobilized to a solid support matrix, which is then used to selectively purify the target protein.

IgG is isolated from serum based on Affinity chromatography on Protein A Agarose. In this technique, the property of Protein A to bind to the Fc region of the Immunoglobulin G molecule is exploited for its purification from serum (Refer Fig 1). Staphylococcal Protein A binds to IgG subclasses of many species and has been widely used to detect and purify IgG. Protein A has varying affinity for IgG of various species (refer table 1). Protein A is isolated from culture supernatant of Staphylococcus aureus and immobilized on cross linked agarose by Cyanogen bromide activation method. The matrix is then used for purification of IgG. Table 1: Relative binding affinity of Protein A to IgG of various species is as follows: Species Relative binding affinity Human ++++ Rabbit ++++ Dog +++ Mouse ++ Chicken-Goat-)

Using this kit, students will carry out purification of IgG from serum using a Protein A Agarose matrix to which the IgG will bind. The IgG will then be eluted from the matrix by reducing the pH. The protein content of the pooled fractions will be checked by absorbance method and the isolated IgG analyzed on 12% SDS PAGE. The purified IgG will be electrophoresed along with the Protein Standard. Post staining, the bands developed will give an indication of the purity of the IgG. The molecular weight of IgG will be determined by comparing its relative mobility with that of the Protein Standard, which has four proteins of known molecular weight: 66 kD, 43 kD, 29 kD and 14.3 kD.

Kit Contents :

- Protein A Agarose Column
- 10X Equilibration Buffer
- 5X Elution Buffer
- 10X Storage Buffer
- Neutralizing Buffer
- Serum Samples
- Protein Standard
- 30% Acrylamide
- 1.5M Tris SDS pH 8.8
- 1 M Tris SDS pH 6.8
- Ammonium persulphate (APS)
- 10X Reservoir Buffer
- Sample Loading Buffer
- Stainer

References :

- Mayers, G. L., & van Oss, C. J. (1998). Affinity Chromatography. Encyclopedia of Immunology
- GODING, J. W. (1996). Affinity Chromatography. Monoclonal Antibodies.

Ordering Information:

Cat. No	PI No.	Product Description
6110600011730	KT106A	GeNei™ Immunoglobulin G Isolation Teaching Kit for 5 expts.

GeNei™ Thin Layer Chromatography Teaching Kit

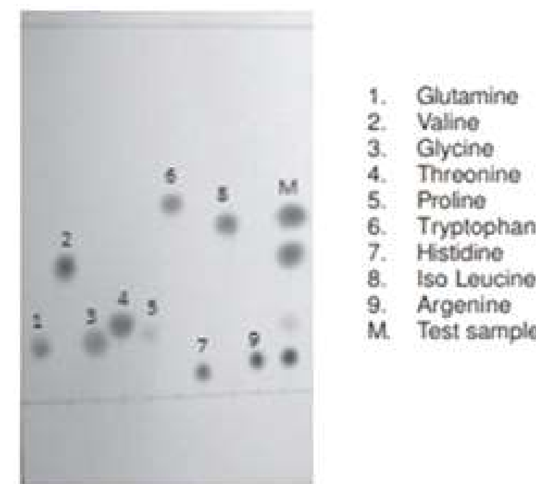
Description :

Thin Layer Chromatography is a method of separating components in a mixture based on their differential affinity for two chemicals, one of which is immobilized ("Stationary Phase") and the other mobile ("Mobile Phase"). As the Mobile Phase travels across a layer of Stationary Phase, it will carry with it the components in the mixture. The components that interact well with the Mobile Phase but poorly with the Stationary Phase, will travel along with the Mobile Phase while those that interact poorly with the Mobile Phase but strongly with the Stationary Phase, will not travel as quickly. When the Mobile Phase is stopped, the components in a mixture would have traveled different distances. TLC is a simple and inexpensive technique that is often used to check the purity of a synthesized compound or to indicate the extent of progress of a chemical reaction. TLC analysis involves a number of steps like preparing a spotting capillary, marking the TLC plate, spotting the TLC plate, developing the TLC plate, drying the plate, visualizing the spots and measuring the Rf values.

Using this kit, students will perform the separation and identification of amino acids in the mixture provided and calculate the Rf values of individual amino acids by using Thin Layer Chromatography. Students will spot and dry the different amino acids along with a test sample on the TLC plate, which will be placed in a TLC chamber for separation using the solvent provided. After migration of the solvent, the Developing Reagent has to be added for visualization of the different amino acids and test sample. The individual components in the Test sample are identified based on their Rf values.

Kit Contents :

- Glutamine
- Valine
- Glycine
- Threonine
- Proline
- Tryptophan
- Histidine
- Iso Leucine
- Arginine
- Test Sample
- TLC plates
- Developing Reagent
- Solution A (Solvent for TLC)
- Solution B (Solvent for TLC)



Amino acid spots developed on a TLC plate.

References :

- Silver, Jack (2020-12-08). "Let Us Teach Proper Thin Layer Chromatography Technique!". Journal of Chemical Education.
- Santiago, Marina; Strobel, Scott (2013-01-01), "Chapter Twenty-Four - Thin Layer Chromatography", in Lorsch, Jon (ed.), Cell, Lipid and Carbohydrate.

Ordering Information:

Cat. No	PI No.	Product Description
6119290021730	KT192	GeNei™ Thin Layer Chromatography Teaching Kit, (with TLC chamber) 10 expts.
6119200021730	KT192A	GeNei™ Thin Layer Chromatography Teaching Kit, 10 expts.

GeNei™ Recombinant Protein Purification Teaching Kit

Description :

Some proteins are present in low abundance in their natural source – cells and tissues of plants and animals or are of high value. In such cases, scientists use Recombinant DNA Technology to develop cells that will produce large quantities of the desired protein, known as expression system. Recombinant expression allows the protein to be tagged for example His-Tag, to facilitate its purification. They can improve the variable yield and poor solubility of many recombinant proteins without altering their conformation or loss of biological activity. Protein tag are peptide sequences genetically grafted on to a recombinant protein. Often this tags are removable by chemical agents or by enzymatic means. Affinity tags are appended to proteins so that they can be purified from their crude biological source using and affinity technique. These include Maltose Binding Protein (MBP) and Glutathione S-Transferase (GST). MBP is a natural affinity tag and can be exploited to facilitate purification of the passenger protein as well as prevent its proteolytice degradation. It also has a remarkable ability to enhance the solubility of its fusion partner.

The Recombinant Protein Purification kit demonstrates the basic principles of purifying recombinant tagged protein. In this kit, approximately 70 kD maltose binding fusion protein is purified using suitable oligosaccharide immobilized on cross linked agarose. The bound fusion protein is eluted using high concentration of a specific sugar. The purified tagged protein is then analyzed by SDS Polyacrylamide gel.

Kit Contents :

- Sample (Lyophilized)
- Column Matrix
- 5X Equilibration Buffer
- 5X Elution Buffer

- Regeneration Buffer
- Protein Standard
- Control MBP
- 30% Acrylamide
- Tris-SDS pH 8.8
- Tris-SDS pH 6.8
- APS
- Sample Loading buffer
- TEMED
- 10X Reservoir Buffer
- Stainer
- Column

References :

- Zachariou, Michael, ed. (2008). Affinity Chromatography: Methods and Protocols (2nd ed.). Totowa, N.J.: Humana Press. pp. 1–2.
- Singh, Naveen K.; DSouza, Roy N.; Bibi, Noor S.; Fernández-Lahore, Marcelo (2015). "Direct Capture of His6-Tagged Proteins Using Megaporous Cryogels Developed for Metal-Ion Affinity Chromatography

Ordering Information:

Cat. No	PI No.	Product Description
6120300011730	Kt203	GeNei™ Recombinant Protein Purification Teaching Kit, 5 expts.

GeNei™ Hydrophobic Chromatography Teaching Kit

Description :

Hydrophobic chromatography is a separation technique that uses the properties of hydrophobicity to separate protein from one another. A chromatography column that is packed with hydrophobic beads is called a hydrophobic interaction matrix. Hydrophobic ("water hating") substances do not mix well with water. When they are dropped into high salt solution they tend to stick together. Side chains of some of the amino acid that make up proteins are very hydrophobic. In the presence of salt, these parts of a protein tend to adsorb to the hydrophobic group on the matrix. High salt causes the three-dimensional structure of the protein to actually change so that the hydrophobic regions of the proteins are more exposed on the surface of the protein and the hydrophilic ("water-loving") regions are more shielded. When the sample is loaded onto the matrix in salt solution, the hydrophobic proteins in the sample will stick to the beads in the column. The more hydrophobic they are the more tightly they will stick. When the salt is removed, the three-dimensional structure of the protein changes again so that the hydrophobic regions of the protein now move to the interior of the protein and the hydrophilic ("water-loving") regions move to the exterior. The result is that the hydrophobic proteins no longer adsorb to the hydrophobic groups on the column matrix and move to the bottom of the column and are thus separated from the other proteins. The main factors influencing hydrophobic interaction chromatography are ligand type, type of base matrix, concentration of salt, pH, temperature and buffer additives.

Using this Kit students will purify Green Fluorescent Protein (GFP) by Hydrophobic Interaction Chromatography (HIC) technique. Green Fluorescent Protein (GFP) is a protein with several stretches of hydrophobic amino acids which results in the total protein being very hydrophobic. When the supernatant, rich in GFP, is loaded on the column in presence of high salt buffer (Binding Buffer) the hydrophobic regions of the GFP adsorb to the column matrix. Other proteins that are less hydrophobic (or more hydrophilic), pass right through the column. This single procedure allows the purification of GFP from a complex mixture of bacterial proteins. The protein of interest is eluted using low salt Elution Buffer, which disrupts the hydrophobic interaction between the GFP and column matrix, causing GFP to let go and "elute" from the column. The eluted protein is observed under ultra violet light for fluorescence

Kit Contents :

- Thiophilic Matrix
- Lysate Samples
- Binding Buffer
- Equilibration Buffer
- Wash Buffer
- Elution Buffer
- Storage Buffer

References :

- Hofstee BH and Otillio NF (1978). Non-ionic adsorption chromatography of proteins. J Chromatogr 159, 57–69. PMID: 649758
- Jennissen HP (1978). Multivalent interaction chromatography as exemplified by the adsorption and desorption of skeletal muscle enzymes on hydrophobic alkyl-agaroses. J Chromatogr 159, 71–83. PMID: 418077

Ordering Information:

Cat. No	PI No.	Product Description
6120200011730	KT202	GeNei™ Hydrophobic Chromatography Teaching Kit, 5 expts

SDS-PAGE Teaching Kit:

Description :

SDS-PAGE is an analytical technique to separate proteins based on their molecular weight. The method is called sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The most used system is also called the Laemmli method after U.K. Laemmli, who was the first to publish a paper employing SDS-PAGE in a scientific study.

Polyacrylamide gels are prepared by mixing acrylamide with bis-acrylamide to form a crosslinked polymer network when the polymerizing agent, ammonium persulfate (APS), is added. TEMED (N,N,N',N'-tetramethylenediamine) catalyses the polymerization reaction by promoting the production of free radicals by APS finally polyacrylamide. In the presence of SDS and a reducing agent that cleaves disulfide bonds critical for proper folding, proteins unfold into linear chains with negative charge proportional to the polypeptide chain length. When subjected to electrophoresis, proteins migrate through the gel strictly according to the size with very little effect from compositional differences. The fact that only microgram quantities of proteins are required, SDS-PAGE has become the most widely used method for determination of molecular mass in a protein sample.

This kit equips students with the knowledge and hands on skills required for SDS-PAGE preparation and analysis of the results.

- To estimate the size of the protein.
- For peptide mapping.
- To compare the polypeptide composition of different structures.
- To estimate the purity of the proteins.
- In Western Blotting and protein ubiquitination.
- To analyse post-translational modifications.

Kit Contents :

- Acrylamide (30%)
- Tris-SDS pH 8.8
- Tris-SDS pH 6.8
- Ammonium persulphate (APS)
- 10X Reservoir Buffer
- Sample Loading Buffer
- Protein Samples
- Protein Marker
- TEMED
- ·Ezee Blue (Gel Stainer)

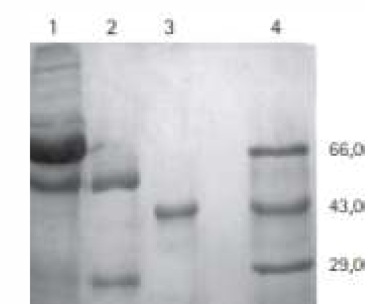


Figure: Purity of protein as determined by SDS-PAGE.

GeNei™

Ordering Information:

Cat. No	PI No.	Product Description
6103000011730	KT30A	GeNei™ SDS-PAGE Teaching Kit, 10 expts.
6103000021730	KT30B	GeNei™ SDS-PAGE Teaching Kit, 20 expts.

GeNei™ 2D-PAGE Teaching Kit

Description :

Two Dimensional Poly-acrylamide Gel Electrophoresis (2D PAGE) is a technique utilizing two parameters of the protein, size and Iso-electric point giving information on thousands of proteins in a single analysis. Separation on the basis of two parameters, usually size and iso-electric point, lowers the probability of two proteins overlapping, and allows greater resolution of proteins. Iso-electric focusing (IEF) gives a measurement of the iso-eletric point (pI) of a protein. The term pI is defined as, the pH at which the proteins net charge is zero, and hence it will not migrate in an electric field. The first dimension also termed as Rod Gel Electrophoresis usually employs iso-electric focusing (IEF) based on pI of the protein. The tube gel is over-laid across the top of the second dimension gel which is SDS-PAGE wherein separation occurs based on the size of proteins.

In this kit, Students will cast the first dimensional gel by using the Rod gel accessories (provided with Cat # 6114500031730) and perform iso-electric focusing of Sample on day 1. Serum is provided as Sample which contains many proteins. The proteins are separated in first dimension according to the pI value of the proteins. Example: Transferrin (pI 6.2) and Immunoglobulin (pI 5.4-8.9). On Day 2, they will cast the second dimensional gel (i.e SDS PAGE) for analyzing the iso-electric focused proteins with Protein Marker. Proteins are separated based on molecular weight. Example: Transferrin (80 kD), Immunglobulin heavy chain (50 kD) and Immunoglobulin light chain (25 kD). The gel is then stained with the stainer.

Kit Contents :

- 2D Boiling Buffer
- 2DSample Loading Buffer (SLB)
- 30% Acrylamide
- Gel Equilibration Buffer
- Ampholyte
- 1.5M Tris-SDS, pH 8.8
- 0.5M Tris-SDS, pH 6.8
- Ammonium per sulphate (APS)
- 10X Reservoir Buffer
- Sample Loading Buffer - SLB (For SDS-PAGE)
- Protein Marker
- Sample 5 vials
- Agarose
- Urea
- Triton X 100
- 5X Anode Buffer
- 5X Cathode Buffer
- TEMED
- Stainer

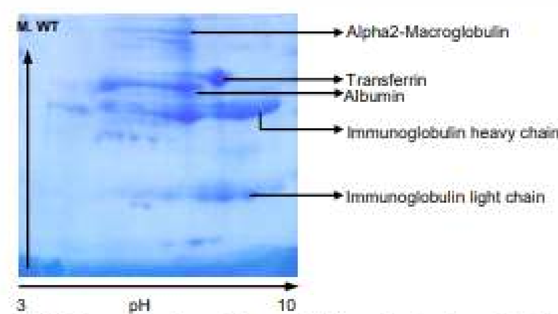


Fig 3: Separation of proteins on a 2 Dimensional polyacrylamide gel.

References :

- O'Farrell, PH (1975). "High resolution two-dimensional electrophoresis of proteins". J. Biol. Chem. 250 (10): 4007–21. doi:10.1016/S0021-9258(19)41496-8.
- Mikkelsen, Susan; Cortón, Eduardo (2004). Bioanalytical Chemistry. John Wiley & Sons, Inc. p. 224

Ordering Information:

Cat. No	PI No.	Product Description
6114500011730	KT145A	GeNei™ 2D-PAGE Teaching Kit, 5 expts.

GeNei™

GeNei™ Enzyme Kinetics Teaching Kit

Description :

Enzyme kinetics is the study of the chemical reactions that are catalysed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction is investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme. Enzymes are usually protein molecules that manipulate other molecules – the enzymes' substrates. These target molecules bind to an enzyme's active site and are transformed into products through a series of steps known as the enzymatic mechanism. These mechanisms can be divided into single-substrate and multiple-substrate mechanisms. Kinetic studies on enzymes that only bind one substrate, aim to measure the affinity with which the enzyme binds this substrate and the turnover rate. Enzyme kinetics can also show the sequence in which these substrates bind and the sequence in which products are released. Although these mechanisms are often a complex series of steps, there is typically one rate-determining step that determines the overall kinetics. This rate-determining step may be a chemical reaction or a conformational change of the enzyme or substrates, such as those involved in the release of product(s) from the enzyme. The activity of an Enzyme is affected by its environmental conditions. Changing these alter the rate of reaction caused by the enzyme. In nature, organisms adjust the conditions of their enzymes to produce an Optimum rate of reaction, where necessary, or they may have enzymes which are adapted to function well in extreme conditions.

In this kit, Horse-Radish Peroxidase (HRP) is provided as a enzyme with its chromogen/substrate - Guaiacol and Hydrogen peroxide. Students will perform the experiments to understand the factors affecting the rate of enzyme – catalyzed reaction namely. Enzyme concentration Substrate concentration Temperature pH

Kit Contents :

- HRP (enzyme)
- Guaiacol
- H2O2
- Assay buffer (pH 3.0)
- Assay buffer (pH 5.0)
- Assay buffer (pH 7.0)
- Assay buffer (pH 9.0)

References :

- David Hames and Nigel Hooper (2005). Biochemistry. Third ed. Taylor & Francis Group: New York.
- Smith, C. M., Marks, A. D., Lieberman, M. A., Marks, D. B., & Marks, D. B. (2005). Marks' basic medical biochemistry: A clinical approach. Philadelphia: Lippincott Williams & Wilkins

Ordering Information:

Cat. No	PI No.	Product Description
6108900011730	Kt89	GeNei™ Enzyme Kinetics Teaching Kit, 5 expts.

GeNei™ Protein Fingerprinting Teaching Kit

Description :

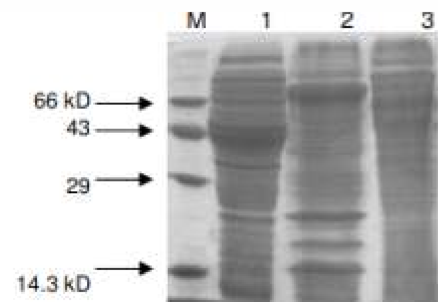
Protein Electrophoresis is one of the key tools of biology research, to visualize and analyze the molecular "fingerprints" of protein content in tissue. Since proteins are encoded by specific DNA sequences, protein fingerprints can reflect genetic similarities as well as differences among organisms. This experiment engages students to think critically about the molecular basis for such similarities and differences, the connection between genes and functions, and the implications of evolutionary relationships among species, from a perspective deeper than outward body forms.

By using Protein Fingerprinting Kit students can analyze proteins from different bacterial sources by

electrophoretic separation of the proteins on a polyacrylamide gel and comparing the banding patterns of prominent proteins between the samples. Students prepare the samples for protein electrophoresis from three bacterial sources provided in the kit. Following electrophoretic separation of the proteins based on their molecular weight, the gel is stained to visualize the proteins and the distribution of proteins known as the Protein Fingerprint. The protein fingerprint of different bacteria is then compared to understand the protein distribution.

Kit Contents :

- Organism - I
- Organism - II
- Organism - III
- Acrylamide Solution
- Tris-SDS (pH-8.8)
- Tris-SDS (pH-6.8)
- Sample Loading Buffer (SLB)
- Protein Marker
- Re-suspension Buffer
- Reservoir Buffer (10X)
- Stainer
- Ammonium persulfate (APS)
- TEMED



Lane M: Protein Molecular Weight Marker
Lane 1: Protein Finger print of Organism I
Lane 2: Protein Finger print of Organism II
Lane 3: Protein Finger print of Organism III

Fig 2: Analysis of the Protein Finger prints from 3 bacterial samples on 12% SDS-PAGE.

References :

- 7(3): 115-124.
- Hjernø K. Protein identification by peptide mass fingerprinting//Mass Spectrometry Data Analysis in Proteomics. Humana Press, 2007: 61-75.

Ordering Information:

Cat. No	PI No.	Product Description
6119600011730	KT196	**GeNei™** Protein Fingerprinting Teaching Kit, 5 expts

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