Bio Bharati Life Science Pvt. Ltd.

Immunoelectrophoresis teaching Kit

Cat# BB-ITK030 (10 Reactions)

Objectives: To learn the technique of immunoelectrophoresis.

Principle: Immunoelectrophoresis is a powerful technique to characterize antibodies. This technique is based on the principle of electrophoresis of antigen for immunodiffusion with a poly-specific antiserum to form precipitin bands.

Electrophoresis: During electrophoresis, molecules placed in an electric field acquire a charge and move towards appropriate electrode. Mobility of the molecules is dependent on a number of factors such as net charge of molecules, size & shape of the molecules, pH of buffer & ionic strength of buffer etc.

Thus, when antigens are subjected to electrophoresis in an agarose gel, they separate according to their acquired charge, size and shape by migrating to different positions.

Immunodiffusion: Antigen thus resolved by electrophoresis is then subjected to immunodiffusion with antiserum added in a trough cut in the agarose gel. Due to diffusion, density gradient of antigen-antibody (Ag-Ab) complex precipitates to form an opaque shaped line in the gel. The precipitin line indicates the presence of antibody specific to a particular antigen.

Duration of experiments: Experiment is carried out over a span of 2 days; approximate time taken an each day is indicated below:

Day1: 3 hours 30 min (electrophoresis & immunodiffusion)

Day2: 30 minutes (observation and interpretation).

Materials required:

Glass ware: conical flask, measuring cylinder.

Reagents: Alcohol, distilled water.

Others: Micropipette, tips, moist chamber **(box with wet cotton).**

Materials provided: The list bellow provides the information about the materials supplied in the kit.

Materials	For 10 reaction	Storage condition
Agarose	2gm	4°C
50X electrophoresis buffer	40ml	4°C
Antigen with Dye	0.2ml	-20°C
Test antiserum(A)	3ml	-20°C
Test antiserum(B)	3ml	-20°C
Gel Puncher & Gel Cutter	1No	RT
Template	2Nos.	RT
Glass Plates	2Nos.	RT

Procedure:

Preparation of gel plate

1. Prepare 10ml of 1.5% agarose (0.15 g/10 ml) in 1X electrophoresis buffer by heating slowly until agarose dissolves completely. Take care not to scorch or froth the solution.

2. Mark the end of a glass plate that will be towards negative electrode during electrophoresis.

3. Place the glass plate on a horizontal surface. Pour and spread 10ml of agarose solution onto the plate. Take care that the plate is not disturbed and allow the gel to solidify.

4. Place the glass plate on the template holder provided and Punch a 3 mm well with the gel puncher as shown in the figure, towards the negative end.

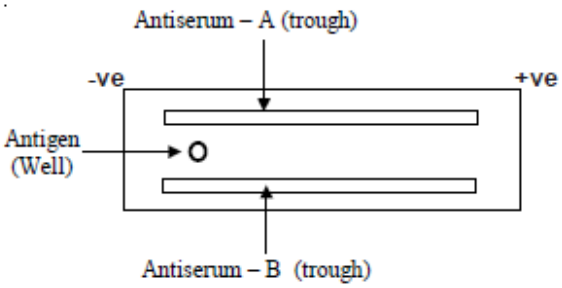
5. Cut two troughs with the gel cutter provided but do not remove the gel from the trough.

Electrophoresis

6. Add 12-15µl of antigen to the well.

7. Place the glass plate in the electrophoresis tank such that the antigen well is at the cathode/negative electrode. Pour 1X electrophoresis buffer such that it covers the gel.

8. Set the voltage to 50-100V and electrophorese until the blue dye travels 3-4cms from the well. Do not electrophorese beyond 3 hours, as it is likely to generate heat.



Immunodiffusion

9. Remove gel from both the troughs and keep the plate at room temperature for 15mins. Add 250µl Antiserum A in one of the troughs and 250µl Antiserum B in the other.

10. Place the plate in a moist chamber and allow diffusion to occur at room temperature, overnight.

Observation:

Observe for precipitin lines between antiserum troughs and the antigen well.

Adapted from internet

Exp. Date: 3 months upon receiving at proper storage condition as mentioned in datasheet.