HiGenoMB™ Unzipping Genes

HIMEDIA®

Product Information

HiPer® Immunoelectrophoresis Teaching Kit

Product Code: HTI005

Number of experiments that can be performed: 5/20

Duration of Experiment: 2 days

Day1- Protocol: 4 hours

Day2- Observations: 15 minutes

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Antiserum A, B and Antigen at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

HiMediaLaboratories™

HiMedia Laboratories Pvt. Limited

A-516, Swastik Disha Business Park, Via Vadhani Indl. Est. LBS Marg, Mumbai - 400 086, India

Tel: 00-91-22-6147 1919 ▪ Fax: 6147 1920, 2500 5764 ▪ Email : info@himedialabs.com ▪ Web : www.himedialabs.com

HIMEDIA®

For life is precious

ISO 9001-2015 CERTIFIED

WHO GMP CERTIFIED

ISO 13485-2012 CERTIFIED

Registered Office :

23, Vadhani Industrial Estate,LBS Marg,
Mumbai - 400 086, India.
Tel. : (022) 4017 9797 / 2500 1607
Fax : (022) 2500 2286

Commercial Office

A-516, Swastik Disha Business Park,
Via Vadhani Indl. Est., LBS Marg,
Mumbai - 400 086, India

Index

Aim:

To learn the technique of Immunoelectrophoresis.

Introduction:

Immunoelectrophoresis is a powerful qualitative technique for the characterization of any antibody. In this method one antigen mixture is electrophoresed in an agarose gel that allows the separation of its different components based on their charge along the gel slide, followed by the lateral diffusion of the serum or monoclonal antibody within the gel. Antibodies specific to the antigens form white precipitation arcs which can be seen against a dark background.
This technique is useful in determining the blood levels of three major immunoglobulins: IgM, IgG and IgA. The process combines antigen separation technique of electrophoresis and immunodiffusion of the separated antigen against an antibody. It is used extensively to check the presence, specificity and homogeneity of the antibodies and can detect relatively high antibody concentrations.

Principle:

In immunoelectrophoresis, the antigen mixture is first electrophoresed to separate its constituents by charge. The antiserum containing the antibodies added into the troughs diffuses with a plane front to react with the antigens. Due to diffusion, density gradient of the antigen and antibody are obtained and at a specific antigen/antibody ratio (equivalence point) huge macromolecules are formed. They form a visible white complex which precipitates as arcs in the gel. The arc is closer to the trough at the point where the antigen is in highest concentration. The method is very specific and highly sensitive because distinct zones are formed. In this method it is important that the ratio between the quantities of antigen and antibody be proper (antibody titer).

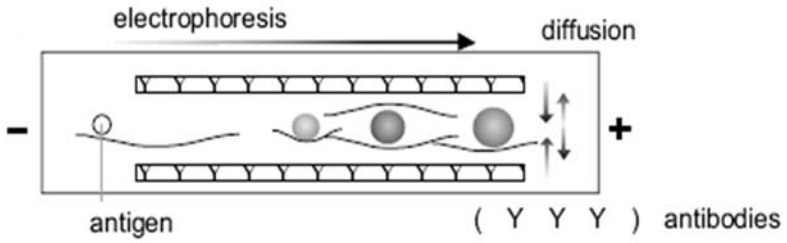

Fig 1: Immunoelectrophoresis technique

Kit Contents:

The kit can be used to perform separation of antigen components and immunodiffusion of the separated antigen against an antibody

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity		Storage
			5 expts	20 expts	
1	MB002	Agarose	0.9 g	3.6 g	RT
2	ML016	50X TAE	40 ml	160 ml	R T
3	TKC100	Antigen	0.06 ml	0.240ml	2-8°C
4	TKC096	Test Antiserum A	0.5 ml	2.0 ml	2-8°C
5	TKC097	Test Antiserum B	0.5 ml	2.0 ml	2-8°C
6	TKC093	Template	1 No.	4 Nos.	RT
7	TKC082	Glass plate	1 No.	4 Nos.	RT
8	TKC083	Gel puncher	1 No.	1 No.	RT

Materials Required But Not Provided:

Glass wares: Conical flask, Measuring cylinder, Beaker
Reagents: Distilled water, alcohol
Other requirements: Incubator (37°C), Microwave or Bunsen burner, Electrophoresis unit, Vortex mixer, spatula, Micropipettes, Tips, Gel cutter, Moist chamber (box with wet cotton)

Storage:

HiPer® Immunoelectrophoresis Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. Store Antiserum A, B and Antigen at 2-8°C. Other kit contents can be stored at room temperature (15-25°C).

Important Instructions:

1. Before starting the experiment the entire procedure has to be read carefully.
2. Always wear gloves while performing the experiment.
3. **Preparation of 1X TAE:** To prepare 300 ml of 1X TAE, add 6 ml of 50X TAE to 294 ml of sterile distilled water.
4. **Preparation of 1.5% Agarose gel:** To prepare 10 ml of agarose gel, add 0.15 g of agarose powder to 10 ml of 1X Electrophoresis Buffer, boil to dissolve the agarose completely.
5. Wipe the glass plates with cotton; make it grease free using alcohol for even spreading of agarose.
6. Cut the well and troughs neatly without rugged margins.
7. Add the antiserum to agarose only after it cools to 55°C as higher temperature inactivates the antibody.
8. Ensure that the moist chamber has enough wet cotton to keep the atmosphere humid.

Procedure:

1. Prepare 10 ml of 1.5% agarose (as given in important instructions).
2. Mark the side of the glass plate that will be towards negative electrode during electrophoresis.
3. Cool the solution to 55-60°C and pour 6 ml/plate on to grease free glass plate placed on a horizontal surface. Allow the gel to set for 30 minutes.
4. Place the glass plate on the template provided.
5. Punch a well with the help of the gel puncher corresponding to the markings on the template at the negative end. Use gentle suction to avoid forming rugged wells.
6. Cut two troughs with the help of the gel cutter, but do not remove the gel from the troughs.
7. Add 10 µl of the antigen to the well and place the glass plate in the electrophoresis tank such that the antigen well is at the cathode/negative electrode.
8. Pour 1X Electrophoresis buffer into the electrophoresis tank such that it just covers the gel.
9. Electrophorese at 80-120 volts and 60-70 mA, until the blue dye travels 3-4 cms from the well. Do not electrophorese beyond 3 hours, as it is likely to generate heat.
10. After electrophoresis, remove the gel from both the troughs and keep the plate at room temperature for 15min. Add 80 µl of antiserum A in one of the trough and antiserum B in the other.
11. Place the glass plate in a moist chamber and incubate overnight at 37°C.

Observation and Result:

Observe for precipitin lines between antiserum troughs and the antigen well (Refer fig 2).

Fig 2: Glass plate showing precipitin lines following immunoelectrophoresis

Note : For better precipitin lines incubate for longer period at 37°C.

Interpretation:

The formation of precipitin line indicates the presence of antibody specific to the antigen.

1. Homogeneity of the antiserum to the antigen is denoted by presence of a single continuous precipitin line
2. Heterogeneity of the antiserum to the antigen is denoted by presence of more than one precipitin line which not only gives an indication of the number of immunodominant epitopes, but also the non identical nature of such epitopes.

Troubleshooting Guide:

Sr.No.	Problem	Probable Cause	Solution
1	No precipitin lines observed	Inadequate filling of the well and troughs	Samples should be loaded directly into the well and troughs without spilling to the sides
		Drying of the agarose gel during incubation	Ensure that the moist chamber has enough moist cotton to avoid drying of the gel
		Insufficient electrophoresis of the antigen	Ensure that the antigen travels at least 3/4th of the gel
2	Blur or improper precipitin lines observed	Samples not loaded properly into the well and troughs	Samples should be loaded directly into the well and troughs without spilling to the sides
		Uneven pouring of gel	Place the glass plate on a flat surface while pouring the gel. Do not disturb the plate once the gel is poured

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at mb@himedialabs.com

Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
23, Vadhani Industrial Estate,
LBS Marg, Mumbai- 86, MS, India

HiMedia Laboratories Pvt. Ltd. Reg. office: 23, Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-61169797 Corporate office: A-516,Swastik Disha Business Park,Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-6147 1919 Email: techhelp@himedialabs.com Website: www.himedialabs.com