HiPer® Counter Current Immunoelectrophoresis Teaching Kit

Product Code: HTI007

Number of experiments that can be performed: 10/20

Duration of Experiment: 2 days

Day1- Protocol: 1 hour

Storage Instructions:

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

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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Aim:

To learn the method of Counter Current Immunoelectrophoresis to rapidly check any antisera for the presence and specificity of antibodies for a particular antigen.

Introduction:

Counter current immunoelectrophoresis is a modification of immunoelectrophoresis in which antigen and antibody migrate towards opposite directions and form a visible white precipitin line in the area between the wells. It is also known as voltage facilitated double immunodiffusion because the migration of antigen and antibody through the agarose gel is due to the applied voltage rather than simple double immunodiffusion. In this method, the antigen and antibody are placed in parallel wells and under the influence of an electric field move towards one another. A precipitin band appears where they meet in the appropriate ratio. This qualitative technique is much faster and more sensitive than the double diffusion technique. The technique was used by Lang and Haan for the detection of antibodies in 1957.

Principle:

In this method, immunoprecipitation occurs when antigen at the cathode (negative pole) is caused to migrate in an electric field through a suitable medium of diffusion against a stream of antibody migrating backward from the anode (positive pole) because of endosmotic flow. When an electrical current is applied through the alkaline buffer, the negatively charged antigen molecules migrate toward the positive electrode and thus towards the wells filled with antibody and the positively charged antibodies are migrated toward the negative electrode. At some point between the wells, a zone of equivalence occurs and the antigen-antibody complex precipitates as a visible white precipitin line.

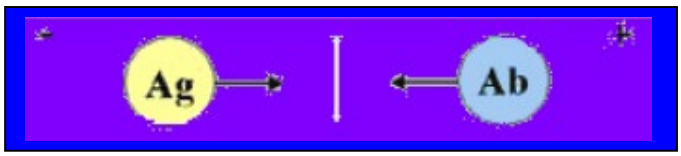

Fig1: Counter Current Immunoelectrophoresis

Kit Contents:

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

Sr. No.	Product Code	Materials Provided	Quantity		Storage
			10 expts	20 expts	
1	MB002	Agarose	1.8 g	3.6 g	RT
2	ML016	50XTAE	72 ml	144 ml	RT
3	TKC046	Positive Control (Antiserum)	0.11 ml	0.22 ml	2-8°C
4	TKC056	Test Antiserum 1	0.11 ml	0.22 ml	2-8°C
5	TKC057	Test Antiserum 2	0.11 ml	0.22 ml	2-8°C
6	TKC066	Test Antiserum 3	0.11 ml	0.22 ml	2-8°C
7	TKC067	Antigen	0.5ml	1.0 ml	2-8°C
8	TKC082	Glass plate	2 Nos.	4 Nos.	RT
9	TKC296	Template	2 Nos.	4 Nos.	RT
10	TKC083	Gel puncher	1 No.	1 No.	RT

Materials Required But Not Provided:

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

Storage:

HiPer® Counter Current Immunoelectrophoresis Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. Store the Antigen, Test Antiserum and Positive Control 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 Electrophoresis Buffer:** 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 TAE, 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. Ensure that the moist chamber has enough wet cotton to keep the atmosphere humid.
   *Molecular Biology Grade Water is recommended (Product code: ML064)

Procedure:

1. Prepare 10 ml of 1.5% agarose (as given in important instructions).
2. Mark the end of the slide that will be towards negative electrode during the 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 wells with the help of gel puncher corresponding to the markings on the template. Use gentle suction to avoid forming rugged wells.
6. Add 10 µl of antigen sample to the wells that will be placed towards the negative electrode and 10 µl of antiserum samples to the wells towards the positive electrode as shown in figure 2

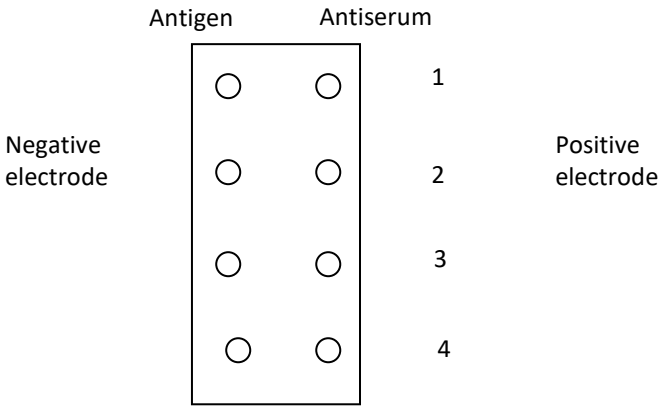

Fig 2: Template for loading of antigen and antiserum onto respective wells

7. Pour 1X TAE into the electrophoresis tank such that it just covers the gel.
8. Electrophorese at 80-120 volts and 55-60 mA, until precipitin lines are observed.
9. Place the glass plate in a moist chamber and incubate overnight at 37°C.

Observation and Result:

Observe for precipitin lines between the antigen and corresponding antiserum wells.

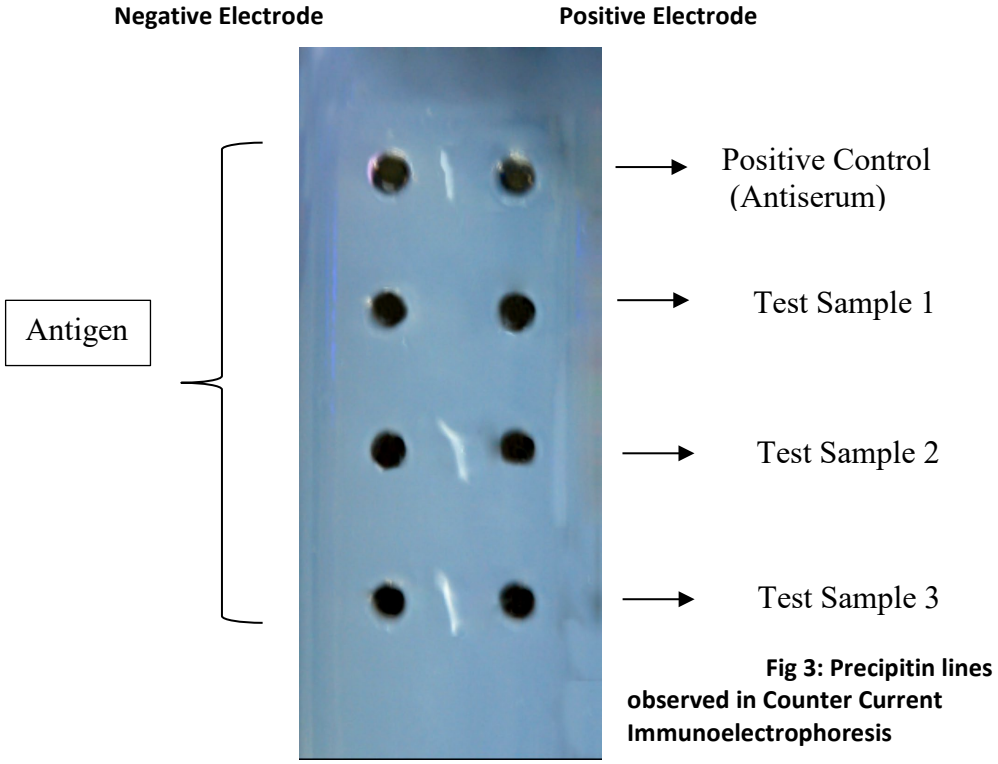

Fig 3: Precipitin lines observed in Counter Current Immunoelectrophoresis

Interpretation:

The precipitin line indicates the presence of antibody specific to the antigen while the absence of precipitin line indicates absence of corresponding antibody in the test antiserum to the given antigen. The presence of more than one precipitin line indicates the heterogeneity of the antibody for the antigen in the test sera.

Troubleshooting Guide:

Sr. No	Problem	Probable Cause	Solution
1	No precipitin lines observed	Inadequate filling of the wells	Samples should be loaded directly into the wells 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
		Reversal of the antigen and antibody wells leading to flow of current in wrong direction	Ensure that the antigen wells are towards the cathode and antibody wells towards the anode during electrophoresis
		Excess buffer added in the electrophoresis tank leads to loss of the samples	The buffer added in the tank should be just enough to have complete contact with the gel and not to immerse the slide completely

2	Blur or improper precipitin lines observed	Samples not loaded properly into the wells	Samples should be loaded directly into the wells 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

Symbol:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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