HiPer® Rocket Immunoelectrophoresis Teaching Kit

Product Code: HTI006

Number of experiments that can be performed: 5/20

Duration of Experiment: 2 days

Day1- Protocol: 2 hours
Day2- Observations: 15 minutes

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Antiserum, Standard and Test Antigens 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 study the technique of Rocket Immunoelectrophoresis for determination of the concentration of antigen in unknown sample.

Introduction:

Rocket Immunoelectrophoresis, also known as electro-immunodiffusion, is a simple, quick and reproducible method for determining the concentration of antigen in an unknown sample. This quantitative one dimensional immunoelectrophoresis method involves a comparison of antigen sample of unknown concentration with a series of dilutions of a known concentration of the antigen and requires a monospecific antibody against the antigen under investigation. In this method, antigen migrates from the well through agarose gel containing antiserum, forming rocket shaped precipitin peaks. The height of this peak is proportional to the concentration of the antigen loaded in the corresponding well.

Principle:

In Rocket Immunoelectrophoresis, negatively charged antigen samples are electrophoresed in an agarose gel containing antibody which is specific to that antigen. As the antigen moves out of the well and enters the agarose gel, it combines with the antibody to form immune complex which is visible as white precipitin arcs. Because the antigen is migrated through the gel under the influence of an applied electric current, it moves in one direction. During the initial phase there is considerable antigen excess over antibody and no visible precipitation occurs. However, as the antigen sample migrates further through the agarose gel, more antibody molecules are encountered that interact with the antigen to form immune complex. When this immune complexes become large enough to be retained within the gel, movement of the antigen stops. The area of precipitin has the shape of a rocket and its height is proportional to the concentration of antigen in the corresponding well.

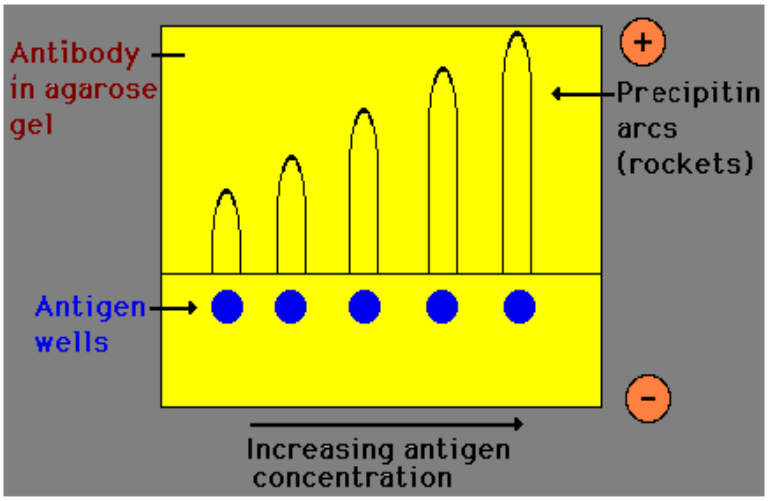


Fig 1: The higher the concentration of antigen loaded in the well, the further it will migrate through the gel before it interacts with sufficient antibody to form precipitin peaks

Kit Contents:

The Kit can be used to perform rocket immunoelectrophoresis to find out the concentration of the unknown antigen samples.

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	ML017	50X TBE	2 x 20 ml	8 x 20 ml	RT
3	TKC047	Antiserum	1.5 ml	6 ml	2-8ºC
4	TKC048	Standard Antigen 1	0.06 ml	0.240 ml	2-8ºC
5	TKC049	Standard Antigen 2	0.06 ml	0.240 ml	2-8ºC
6	TKC050	Standard Antigen 3	0.06 ml	0.240 ml	2-8ºC
7	TKC051	Standard Antigen 4	0.06 ml	0.240 ml	2-8ºC
8	TKC052	Test Antigen A	0.06 ml	0.240 ml	2-8ºC
9	TKC053	Test Antigen B	0.06 ml	0.240 ml	2-8ºC
10	TKC410	Template	1 No	4 No	RT
11	TKC054	Glass plate	1 No.	4 Nos.	RT
12	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 (37ºC), Microwave or Bunsen burner, Vortex mixer, spatula, Micropipettes, Tips, Moist chamber (box with wet cotton)

Storage:

HiPer® Rocket Immunoelectrophoresis Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. Store Antiserum, Standard and Test Antigens at 2-8ºC. Other reagents can be stored at room temperature (15-25ºC).

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 TBE:** Remove the 20 ml tablet of 50X TBE from the pouch into a 250 ml glass beaker. Add 20 ml of sterile distilled water to the beaker and heat in a microwave for 3-5 seconds till the tablet turns into the liquid form. Take the 50X TBE solution into a 1000 ml cylinder, rinse the beaker with sterile distilled water to collect and pour it into the cylinder and make up the volume to 1000 ml with sterile distilled* water to get 1X TBE buffer.
4. **Preparation of 1% Agarose gel:** To prepare 15 ml of agarose gel, add 0.15 g of agarose powder to 15 ml of 1X TBE Buffer, boil to dissolve the agarose completely.
5. Add the antiserum to agarose only after it cools to 55°C. Higher temperature will inactivate the antibody.
6. Wipe the glass plates with cotton; make it grease free using alcohol for even spreading of agarose.
7. Cut the wells neatly without rugged margins.
8. 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 15 ml of 1 % agarose (as given in important instructions).
2. Cool the solution to 55-60ºC and add 250 µl of antiserum to 13 ml of agarose solution. Mix well for uniform distribution of antibody.
3. Pour agarose solution containing the antiserum on to a 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. Use gentle suction to avoid forming rugged wells.
6. Add 10 µl of the given standard antigen and test antigen samples to the wells.

    A. Standard Antigen A (1.87 mg/ml)
    B. Standard Antigen B (0.94 mg/ml)
    C. Standard Antigen C (0.47 mg/ml)
    D. Standard Antigen D (0.23 mg/ml)
    E. Test Antigen 1
    F. Test Antigen 2

7. Pour 1X TBE buffer into the electrophoresis tank such that it just covers the gel.
   Note: The remaining 1X TBE buffer can be stored at room temperature.
8. Electrophorese at 80-120 volts and 60-70 mA, until the blue dye travels 3-4 cm from the well. Do not electrophorese beyond 3 hours, as it is likely to generate heat.
9. Incubate the glass plate in a moist chamber overnight at 37ºC.

Observation and Result:

Observe for precipitin peaks in the shape of 'Rocket' formed against a dark background.
Mark the tip of the precipitin peaks and measure the peak height from the upper edge of the well to the tip of the peak as shown in table 2.

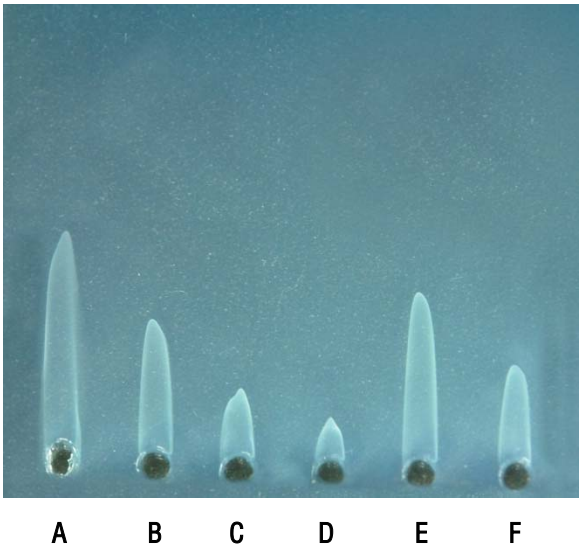
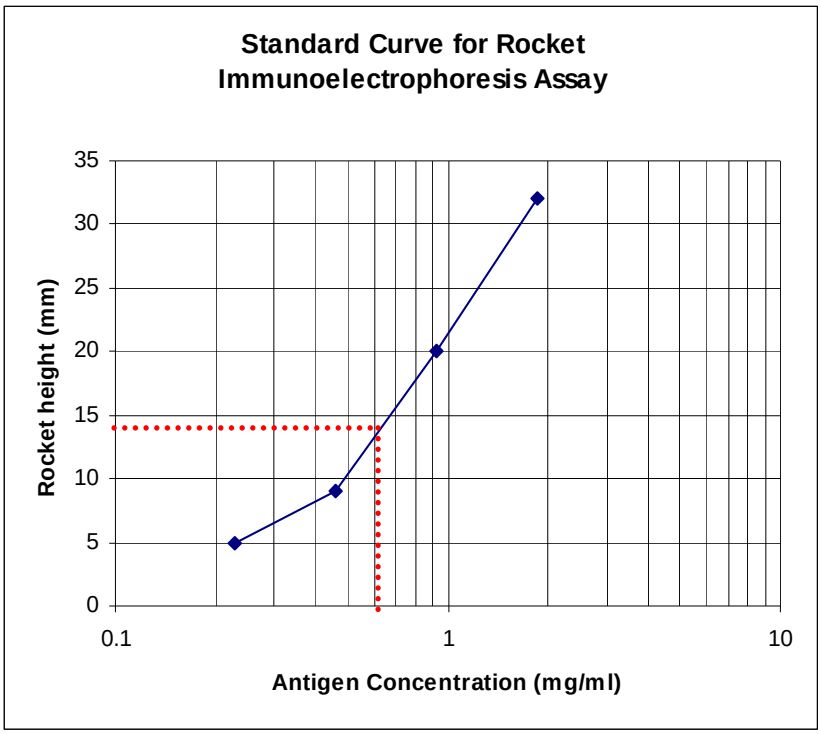


Fig 2: Precipitin peaks (Rockets) observed in Rocket Immunoelectrophoresis

Table 2: Results of Rocket Immunoelectrophoresis

Sample	Standard Antigen Concentration (in mg/ml)	Rocket height (in mm)
A	1.87	
B	0.94	
C	0.47	
D	0.23	
E	Test Antigen 1	
F	Test Antigen 2	

Plot a graph of the rocket height (on Y-axis) versus the concentration of antigen (on X-axis) on a semi-log graph sheet. Determine the concentration of the unknown from the graph by finding the concentration against the rocket height.



Interpretation:

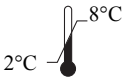
The height of the precipitin peak depends on the concentration of antigens loaded in the corresponding wells. By plotting the graph of concentration of antigens versus length of the precipitin peaks one can calculate the concentration of test antigen.

Troubleshooting Guide:

Sr. No.	Problem	Probable Cause	Solution
1	No precipitin peaks observed	Inadequate filling of the wells	Sample should be loaded directly into the well 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
		Inactivation of antiserum	Antiserum should be added to the agarose gel only after the temperature reaches to 55-60ºC
2	Blur precipitin peaks observed	Inactivation of antiserum	Antiserum should be added to the agarose gel only after the temperature reaches to 55-60ºC
		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

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