Bio Bharati Life Science Pvt. Ltd.

Rocket Immunoelectrophoresis Teaching Kit
Cat# BB-ITK040 (10 Reactions)

Objective: To perform Rocket Immuno-electrophoresis (RIEP) for the determination of various concentrations of antigen in given unknown sample.

Principle: Rocket Immunoelectrophoresis (RIEP) also known, as electro-immuno diffusion is a simple, quick and reproducible method for determining the concentration of antigen (Ag) in an unknown sample. Various concentrations of antigen are loaded side by side in small circular wells along the edge of an agarose gel that contains the specific antibody (Ab). On electrophoresis, the antigen begins to migrate towards the anode and interacts with antibody molecules to form a soluble antigen-antibody (Ag-Ab) complex. However, as the samples electrophorese farther through the gel, more antibody molecules are encountered which interact with the antigen and when the "**equivalence point**" is reached, the Ag-Ab complex precipitates. This precipitin line is visible in the form of a rocket.

Higher the amount of antigen loaded in the well, farther the antigen will travel through the gel. Hence, with increasing antigen concentration, a series of rockets of increasing heights are visible that is proportional to amount of antigen in the well. Therefore, a direct measurement of the height of rocket reflects the corresponding antigen concentration. A standard graph of antigen concentration versus peak height is then plotted and from the peak height of the unknown sample, concentration of antigen is determined.

Kit Description: A standard antigen is supplied at four different concentrations along with two different concentrations of

test antigen. Antigen samples will be electrophoresed on an agarose gel containing the corresponding supplied antiserum and observed for formation of rocket.

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

Materials	For 10 reactions	Store at
Agarose	**3gm**	**RT**
50X electrophoresis buffer	**30ml**	**4°C**
Antiserum	**5ml**	**-20°C**
Standard antigen with Dye (A/B/C/D)*	**0.20ml each**	**-20°C**
Test antigen (1&2) with dye.	**0.10ml each**	**-20°C**
Gel Puncher, Glass Plates & Templates	**1no, 2nos, & 2nos respectively**	**RT**

***Standard antigens of the following concentration have been provided:

Sample No.	Std. Conc. (mg/ml)
A	**0.125**
B	**0.25**
C	**0.5**
D	**1.0**

Materials required:

Glass ware: Conical flask, measuring cylinder.

Reagents: Alcohol, distilled water.

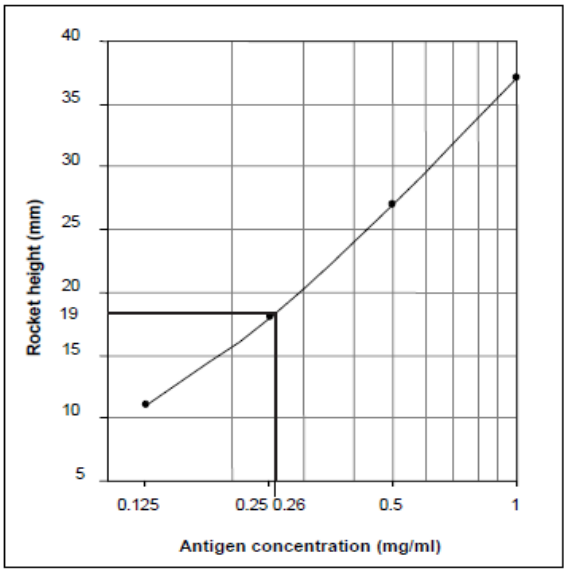
Others: Micropipette, tips

Procedure:

1. Prepare 8ml of 1.0% agarose (0.1 g/10 ml) in 1X electrophoresis buffer by heating slowly until agarose dissolves completely. Take care not to scorch or froth the solution.
2. Allow the molten agarose to cool down to 55°C.
3. Add 0.4ml of antiserum to 8ml of agarose solution and mix gently to ensure uniform distribution of antiserum.
4. Pour the mixture onto a glass plate placed on a horizontal surface and allow it to solidify.
5. Place the glass plate on the template holder and punch 3mm wells towards one edge of the plate.
6. Place the glass plate in the electrophoresis tank; ensure that the wells are towards the cathode. Fill the tank with 1X electrophoresis buffer till it covers the gel. Connect the power cord to the electrophoretic power supply according to the convention: Red: anode and Black: cathode.
7. Add 10µl each of the given standard antigen and test antigen into the wells. Loading of wells should be carried out quickly to minimize diffusion from the well.
8. Electrophorese the samples at 100 volts, till the rockets are visible or the dye front reaches the edge. Then stop electrophoresis, remove the glass plate from the electrophoresis tank.
9. Observe the precipitation peak or rocket formed against a dark background. If the rockets are not clearly visible, incubate the plate in a moist chamber at room temperature for overnight.
10. Measure the rocket height (from upper edge of the well to tip of the rocket) and construct a standard graph by plotting it on Y-axis (linear scale) against the concentration of antigen on X-axis (log scale) on a semi-log graph sheet.
11. Determine the concentration of test antigen sample from the standard curve against the corresponding rocket height.

For example, if following are the results obtained for an RIEP assay, plot the graph as given below

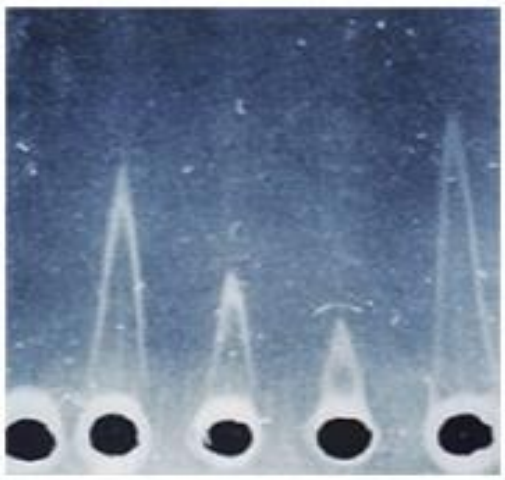
Sample no.	Standard concentration (mg/ml)	Rocket height (in mm)
A	0.125	11
B	0.25	18
C	0.5	26
D	1.0	38
Test (1/2)	---------	19



From the graph, height of 19mm corresponds to a concentration of 0.26mg/ml. Therefore, antigen in e test sample is =0.26mg/ml.

Observation:

Formation of rockets will be as follows



Result: From the standard curve, determine thee unknown concentration of antigen.

Adapted from internet