



Radial Immunodiffusion
Cat No# BB-ITK010 (10 Reactions)

Objective: To study the immunodiffusion technique by Single Radial Immunodiffusion.

Principle: Single Radial Immunodiffusion (RID) is used extensively for the quantitative estimation of antigens. The antigen antibody precipitation is made more sensitive by the incorporation of antiserum in the agarose. Antigen (Ag) is then allowed to diffuse from wells cut in the gel in which the antiserum is uniformly distributed. Initially, as the antigen diffuses out of the well, its concentration is relatively high and soluble antigen-antibody adducts are formed. However, as Ag diffuses farther from the well, the Ag-Ab complex reacts with more amount of antibody resulting in a lattice that precipitates to form a precipitin ring. The diameter of the precipitin ring is proportional to the concentration of antigen. With increasing concentration of antigen, precipitin rings with larger diameter are formed.

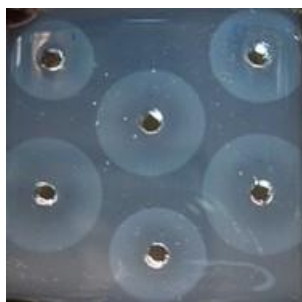


Fig 1: Standard RID Assay

Thus, by running a range of known antigen concentrations on the gel and by measuring the diameters of their precipitin rings, a standard graph is plotted. Antigen concentrations of unknown samples, run on the same gel can be found by measuring the diameter of precipitin rings and extrapolating this value on the standard graph.

Duration of the experiment: Experiment is carried out over a span of 2 days, approximate time taken on each day is indicated below:

Day 1: 1 hour (Preparation of gel and loading of antigen samples).

Day 2: 30 minutes (Observation and Interpretation).

Materials Required:

Glassware: Conical flask, Measuring cylinder.

Reagents: Alcohol, Distilled water.

Other Requirements: Micropipette, Tips, Moist chamber (box with wet cotton).

Materials Provided:

The list below provides information about the materials supplied in the kit. The products should be stored as suggested. Use the kit within 6 months of arrival.

Materials	Quantity (10 Experiments)	Store
Agarose	1gm	4°C
10X Assay Buffer	10ml	4°C
Standard Antigen (D)	0.23ml	-20°C
Test Antigen (1)	0.2ml	-20°C
Antiserum	2ml	-20°C
Gel puncher	1 No	RT
Template	2 Nos.	RT
Glass plates	2 Nos.	RT

1. Standard Antigen A (0.25 mg/ml)
2. Standard Antigen B (0.5 mg/ml)
3. Standard Antigen C (1.0 mg/ml)
4. Standard Antigen D (2.0 mg/ml)
5. Test Antigen – 1
6. Test Antigen – 2

[Note: Standard Antigen D (2.0 mg/ml) and Test Antigen-1 samples are supplied. Prepare C, B and A standard antigen samples starting from D by serial dilutions and Test Antigen-2 from Test Antigen-1 by double dilution.]



Procedure:

1. Prepare 6ml of 1.5% agarose (0.09 g/6 ml) in 1X assay buffer by heating slowly till agarose dissolves completely. Do not scorch or froth the solution.
2. Allow the molten agarose to cool down to 55°C.
3. Add 100-120µl of antiserum to 6ml of agarose solution. Mix gently by swirling for uniform distribution of antibody.
4. Pour agarose solution containing the antiserum onto a grease free glass plate set on a horizontal surface. Leave it undisturbed to form a gel.
5. Cut wells using a gel puncher as shown in figure bellow using the template provided.
6. Add 10 µl of the given standard antigens and test antigens to the wells as shown.

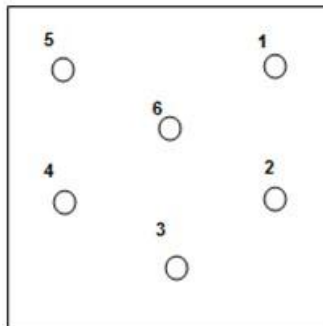


Fig 2: Pattern of addition of standard antigen and test antigen samples to wells

7. Keep the gel plate in a moist chamber (box containing wet cotton) and incubate overnight at room temperature.
8. Mark the edges of the circle and measure the diameter of the ring. Note down your observations as in table 1.
9. Plot a graph of diameter of ring (on Y-axis) versus concentration of antigen (on X-axis) on a semi-log graph sheet.
10. Determine concentration of unknown by reading the concentration against the ring diameter from the graph.

Adapted from internet

For e. g., if the following results are obtained for an RID assay, plot the graph as shown in figure 3.

Table 1: Result of RID assay

Sample No.	Std. Ag conc. (mg/ml)	Ring diameter (mm)
A	0.25	6
B	0.5	8
C	1.0	10
D	2.0	12
Test Sample 1		11

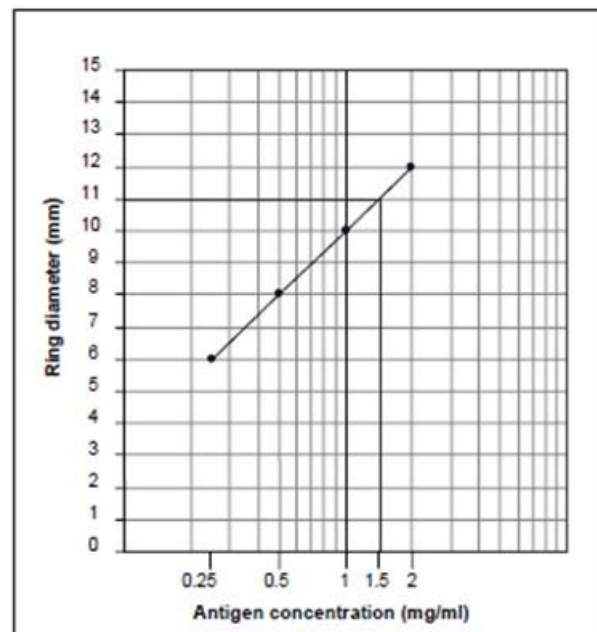


Fig 3: Standard Curve for RID Assay

From the graph, the concentration of test sample is **1.5mg/ml**.

Interpretation: The diameter of the precipitin ring depends upon the concentration of antigens loaded in the wells. By plotting the graph of concentration of antigens versus diameter of the corresponding precipitin ring one can calculate the concentration of any test antigen.

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