Radial Immunodiffusion Teaching Kit GeNei™

Objectives:

To learn the technique of **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 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.** (Refer fig.1).

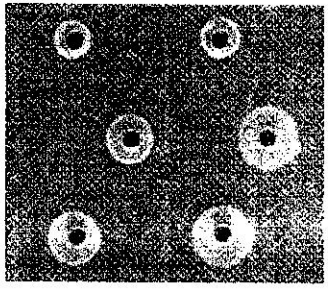

Fig.1: Standard RID Assay

By loading a range of known antigen concentrations on the gel and by measuring the diameters of their precipitin rings, a calibration graph is plotted. Concentrations of unknown antigens can be determined by measuring the diameter of precipitin rings and extrapolating this value on the calibration graph.

Kit Description:

In this kit, Standard Antigen at four different concentrations (0.25 mg/ml, 0.5 mg/ml, 1.0 mg/ml, and 2.0 mg/ml) are supplied along with two test antigen samples. These samples are allowed to diffuse in an agarose gel containing Antiserum. Diameter of precipitin ring thus formed will be measured and plotted against the Standard Antigen concentrations to obtain a standard curve. Using this, the concentration of two Test Antigens will be determined.

6101000011730: The kit is designed to carry out 15 Radial Immunodiffusion Experiments

Duration of 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 Provided:

The list below provides information about the materials supplied in the kit. The products should be stored as suggested.

Note: Sample of semi-log graph sheets are provided with the manual. Photocopy as required

Materials	Quantity 6101000011730 (15 Expts.)	Store
Agarose	2 g	4°C
10X Assay Buffer	20 ml	4°C
Standard Antigens (A, B, C & D)	0.35 ml each	4°C
Test Antigen (1 & 2)	0.35 ml each	4°C
Antiserum	2 ml	4°C
Gel Punch with syringe	1 No.	4°C
Glass Plate	5 Nos.	4°C
Template	2 Nos.	4°C

***Standard Antigens, Test Antigen and Antiserum are supplied in solution form.**

Materials Required:

Glassware: Conical flask, Measuring cylinder.
Reagents: Alcohol, Distilled water.
Other Requirements: Micropipette, Tips, Moist chamber (box with wet cotton

Note:

- **Read the entire procedure prior to starting the experiment.**
- Dilute required amount of 10X assay buffer to 1X with distilled water.
- Wipe the Glass Plate with alcohol thoroughly to make it grease free for even spreading of agarose.
- Glass plates supplied can be used for the next set of experiments after proper cleaning as mentioned above.
- Cut the wells neatly without rugged margins to get a perfect ring of precipitation.
- Add the Antiserum to agarose only after it cools to 55°C. Higher temperature will inactivate the antibody.
- Assay buffer: Phosphate buffered saline.

Procedure:

1. Prepare 10 ml of 1.0% agarose (0.1 g/10 ml) in 1X Assay Buffer by heating slowly till agarose dissolves completely. Take care not to scorch or froth the solution.
2. Allow the molten agarose to cool to 55°C.
3. Add 120 µl of antiserum to 6 ml of agarose solution. Mix by gentle 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 for 15 minutes to solidify.
5. Cut wells using a Gel Puncher as shown in figure 2, using the Template provided.
6. Add 20 µl of the given Standard Antigens and Test Antigens to the wells as shown in figure 2.

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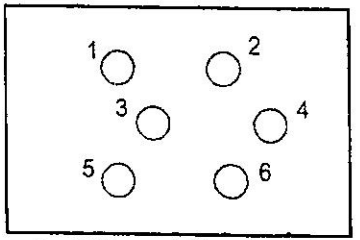

Fig. 2: Pattern of addition of Standard Antigen and Test Antigen samples to wells

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

7. Keep the gel plate in a moist chamber (box containing wet cotton / tissues) 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 the concentration of unknown by reading the concentration against the ring diameter from the graph

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

Sample No.	Std. Ag Conc. (in mg/ml.)	Ring Diameter (in mm)
A	0.25	6
B	0.5	8
C	1.0	10
D	2.0	12
Test Antigen 1	1.5	11
Test Antigen 2	0.7	9

Table 1: Results of RID Assay

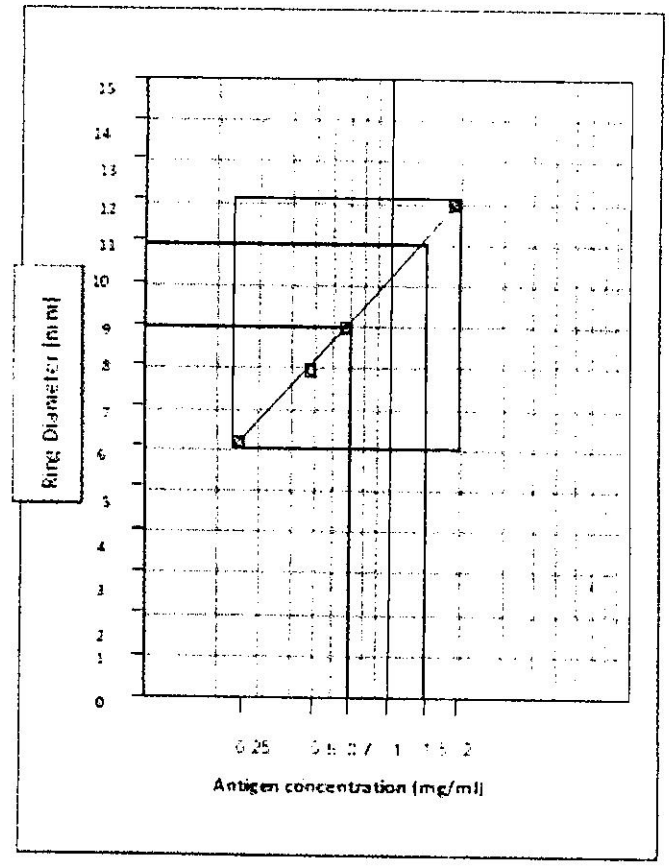

Fig. 3: Standard Curve for RID Assay

From the graph,
Concentration of antigen in Test Antigen 1=1.5 mg/ml
Concentration of antigen in Test Antigen 2=0.7 mg/ml

Result:

From the standard curve, determine and report concentration of antigen in the test samples

Ordering Information:

Product	Size	Cat #
GeNei™ Radial Immunodiffusion (RID) Teaching Kit (Consumables for 15 Experiments)	1 EA	6101000011730

Email:
Sales: sales@geneilabs.com

Customer Support: techsupport@geneilabs.com

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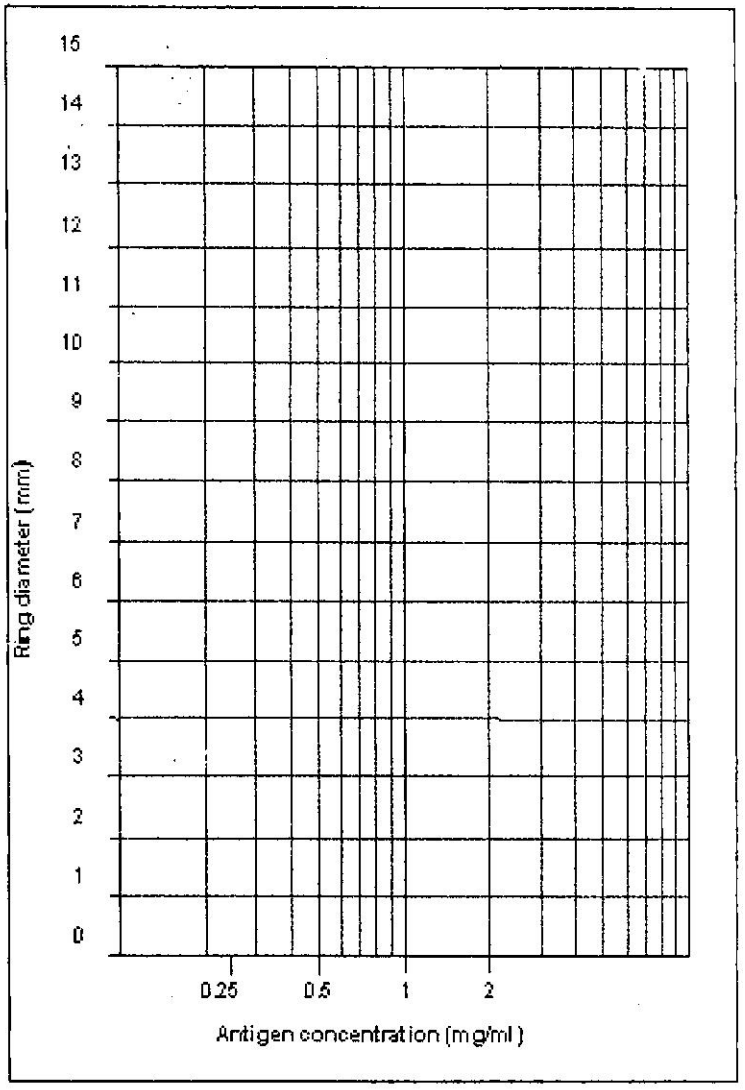
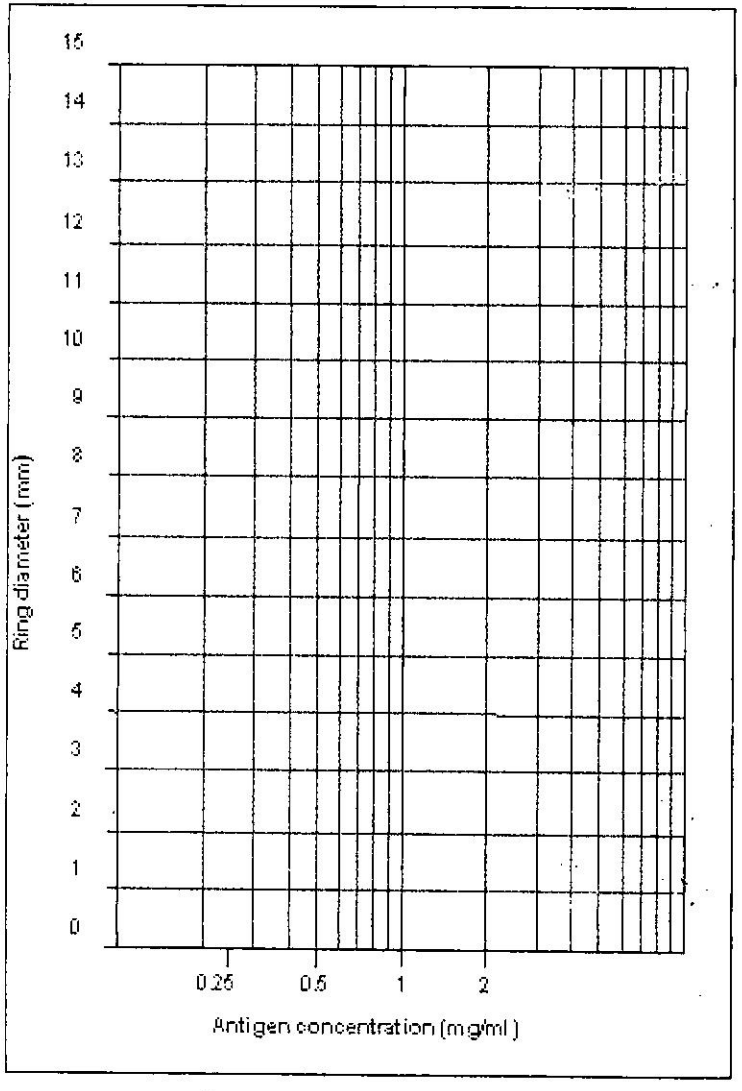


Standard Curve for RID Assay



Standard Curve for RID Assay

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Notes :