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SN Radial Immunodiffusion Teaching Kit

Solution Based

Innovation Speaks

SN Radial Immunodiffusion Teaching Kit

Product Code: SNLS - TK115

Number of experiments that can be performed : 20

Duration of Experiment : 2 days

Day 1- Protocol: 1 hour

Day 2- Observations: 30 minutes

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store the 10X Assay buffer, Antiserum and Antigens at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To study the immunodiffusion technique by Single Radial Immunodiffusion.

Introduction:

Single Radial Immunodiffusion, also known as Mancini technique, is a quantitative immunodiffusion technique used to detect the concentration of antigen by measuring the diameter of the precipitin ring formed by the interaction of the antigen and the antibody at optimal concentration. In this method the antibody is incorporated into the agarose gel whereas the antigen diffuses into it in a radial pattern. Thus the antibody is uniformly distributed throughout the gel.

Principle:

Single Radial Immunodiffusion is used extensively for the quantitative estimation of antigen. Here the antigen-antibody reaction is made more sensitive by the addition of antiserum into the agarose gel and loading the antigen sample in the well. As the antigen diffuses into the agarose radially in all directions, its concentration continuously falls until the equivalence point is reached at which the antigen concentration is in equal proportion to that of the antibody present in the agarose gel. At this point ring of precipitation ('precipitin ring') is formed around the well. 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.

The size of the precipitin rings depend on

- Antigen concentration in the sample well
- Antibody concentration in the agarose gel
- Size of the sample well
- Volume of the sample

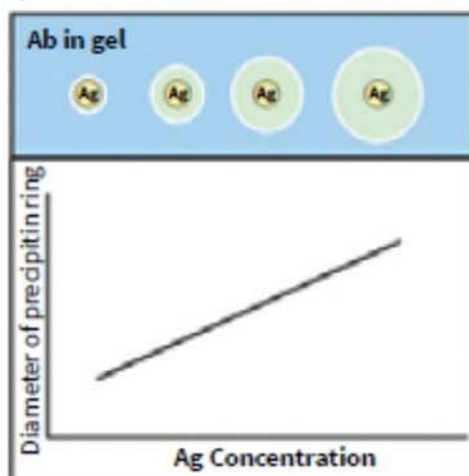


Fig 1: In Single Radial Immunodiffusion assay the diameter of the precipitin ring increases with increasing concentration of the antigen

Thus, by having various concentrations of a standard antigen, standard curve can be obtained from which one can determine the amount of an antigen in an unknown sample. Thus, this is a quantitative test. If more than one ring appears in the test, more than one antigen/antibody reaction may have occurred. This could be due to a mixture of antigens or antibodies.

This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient samples.

Kit Contents:

The Kit can be used to perform single radial Immunodiffusion technique.

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

Sr. No.	Materials Provided	Quantity	Storage
		20 expts	
1	Agarose	2.3 g	R T
2	10X Assay buffer	23 ml	2-8 °C
3	Antiserum	1.8 ml	2-8°C
4	Standard Antigen A	0.24 ml	2-8°C
5	Standard Antigen B	0.24 ml	2-8°C
6	Standard Antigen C	0.24 ml	2-8°C
7	Standard Antigen D	0.24 ml	2-8°C
8	Test Antigen 1	0.24 ml	2-8°C
9	Test Antigen 2	0.24 ml	2-8°C
10	Glass plate	4 Nos.	R T
11	Gel puncher	1 No.	R T
12	Template	4 Nos.	R T

Materials Required But Not Provided:

Glass wares: Conical flask, Measuring cylinder, Beaker

Reagents: Distilled water, alcohol

Other requirements: Incubator (37°C), Microwave or Bunsen burner, Vortex mixer, spatula, Micropipettes, Tips, Moist chamber (box with wet cotton)

Storage:

SN Radial Immunodiffusion Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. Store the Assay buffer, Antiserum, Standard and Test antigens 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 Assay Buffer:** To prepare 10 ml of 1X Assay Buffer, add 1 ml of 10X Assay buffer to 9 ml of sterile distilled water*.
4. **Preparation of 1% Agarose gel:** To prepare 10 ml of Agarose gel, add 0.1 g of Agarose powder to 10 ml of 1X Assay Buffer, 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 wells neatly without rugged margins.
7. Add the antiserum to agarose only after it cools down to 55°C as higher temperature will inactivate the antibody.
8. Ensure that the moist chamber has enough wet cotton to keep the atmosphere humid.
9. Ensure that the slide is grease free before pouring the gel.

* Molecular biology grade water is recommended .

Procedure:

1. Prepare 10 ml of 1% agarose gel (as give in the important instructions). Take 6 ml of this gel solution in a clean test tube.
2. Allow the solution to cool down to 55-60°C and add 80 μ l of antiserum to 6 ml of agarose solution. Mix well for uniform distribution of the 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 corresponding to the markings on the template. Use gentle suction to avoid forming rugged wells.
6. Add 10 μ l of the given standard antigen and test antigen samples to the wells.
 - A. Standard Antigen A (3.75 mg/ml)
 - B. Standard Antigen B (7.5 mg/ml)
 - C. Standard Antigen C (15 mg/ml)
 - D. Standard Antigen D (30 mg/ml)
 - E. Test Antigen 1
 - F. Test Antigen 2

E	F
A	B
C	D

Fig 2: Template of pattern of wells for loading of standard and test antigens

7. Incubate the glass plate in a moist chamber overnight at 37°C.

Observation and Result:

Observe for precipitin rings surrounding the antigen wells (Fig 3). Mark the edges of the precipitin rings and measure the diameter of the rings as shown in table 2.

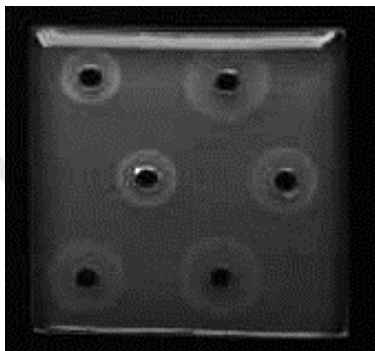
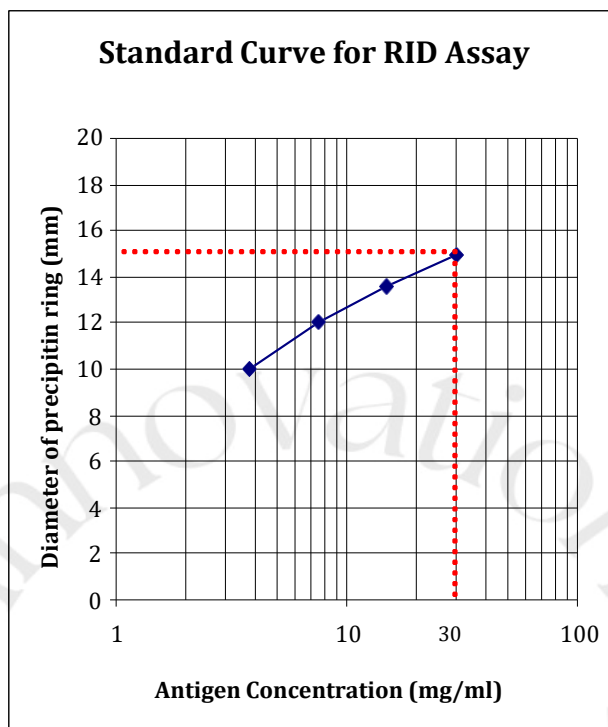


Fig 3: Precipitin rings observed in Single Radial Immunodiffusion

Table 2: Results of Single Radial Immunodiffusion

Sample	Standard Antigen Concentration (in mg/ml)	Ring Diameter (in mm)
A	3.75	
B	7.5	
C	15.0	
D	30.0	
E	Test Antigen 1	
F	Test Antigen 2	

Plot a graph of the diameter of the precipitin ring (on Y-axis) versus the concentration of antigen (on X-axis) on a standard graph sheet. Determine the concentration of the unknown antigen from the graph by finding the concentration against the ring diameter.



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.

Troubleshooting Guide:

Sr.No	Problem	Probable Cause	Solution
1	No precipitin ring 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 55-60°C
2	Blur precipitin ring observed	Inactivation of antiserum	Antiserum should be added to the agarose gel only after the temperature reaches to 55-60°C
		Uneven pouring of gel	Place the glass plate on a flat surface while pouring the gel. Do not move the plate once the gel is poured



**SHANKARANARAYANA
LIFE SCIENCES**
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— SN Research Unit



SN Manufacturing Unit



SN Diagnostic Unit

Contact Us -

☎ +91- 63613 81129 / 99005 05076

🌐 www.snlifesciences.in

✉ sales@snlifesciences.com / drmondal@snlifesciences.com

🏠 No.57/90, 3rd Road, 4th Phase Bommasandra Industrial Area,
Bengaluru - 560099

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