

USER MANUAL

Single Radial Immunodiffusion Kit

Catalog No: **IMI-KIT-1011a** - for **5** experiments
IMI-KIT-1011b - for **10** experiments
IMI-KIT-1011c - for **20** experiments

Experiment duration – 1:30 hours

**Single Radial Immunodiffusion Kit contains
Standard reagents
and
Protocol**



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Single Radial Immunodiffusion Kit

I. PRINCIPLE

The Immune system is a remarkably versatile defense system that has evolved to protect animals from invading pathogenic microorganisms and cancer. It is able to generate an enormous variety of cells and molecules (antibody) capable of specifically recognizing and eliminating a variety of foreign invaders (antigen).

Antigen and antibody interaction is the fundamental reaction of immunology. These interactions are useful in the defense of the body against bacterial and viral infections and toxins. The defense capabilities are dependent upon the recognition of antigens by humoral components of the immune system. Specific antibodies are then produced in response to exposure to the antigen. The binding of an antibody with an antigen results in the formation of large macromolecular complex. These complexes form precipitates, which are useful for laboratory and diagnostic tests. Precipitation reactions of antibodies and antigens in agarose gels provide a method of analyzing the various antibody-antigen reactions in a system. When antibodies and antigens are inserted into different areas of an agarose gel, they diffuse toward each other and form opaque bands of precipitate at the interface of their diffusion fronts.

Radial Immunodiffusion (RID) Assay is a specialized form of immunodiffusion in which antibody is incorporated into molten agarose, which is poured into a petridish and allowed to solidify. Small wells are cut into the agarose gel and are filled with known concentrations of antigen, which corresponds to the antibody in the agarose. Samples of unknown concentrations are placed in similar wells. The antigens in solution then diffuse outwards from the well in a circular precipitate ring surrounding the well. Generally it takes 24 to 48 hours for optimal diffusion to occur and precipitation to become apparent. The diameter of the precipitin ring is proportional to the concentration of the antigen present in the test sample. By comparing the diameter of the test specimen precipitin ring to known standards, a relatively less sensitive estimation of the concentration of specific antigen can be achieved.

II. OVERVIEW

Radial immunodiffusion is a reliable quantitative method and is particularly useful for difficult samples, e.g., that are turbid and for which other methods are inappropriate. This technique detects the quantity of antigen by measuring the radius surrounding samples of the antigen, marking the boundary between it and antibody. As the antigen diffuses into the gel, it reacts with the antibody and when the equivalence point is reached a ring of precipitation is formed. This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient samples.

III. ADVANTAGES

- This kit helps the student to understand the principle and basic function of the technique. It can be used to determine the antigen quantitatively.
- The technique is relatively simple, rapid to perform and of low cost because it requires no special equipment.
- It contains standard reagents and protocol for better results.

IV. KIT COMPONENTS AND STORAGE

All the reagents should be stored at 2-8°C when not in use.

SI No	Components	Quantity		
		5 expts	10 expts	20 expts
1	Agarose	0.75 g	1.5 g	3 g
2	5X Assay buffer	25 ml	50 ml	100 ml
3	Antiserum	2.5 ml	4.5 ml	9 ml
4	Standard Antigen	0.5 ml	1 ml	2 ml
5	Test Antigen	200 µl	400 µl	800 µl

Materials required (not included in the kit):

Glassware: Conical flask, Measuring cylinder.

Reagents: Alcohol, Distilled water.

Other Requirements: Micropipette, Tips, Moist chamber (box with wet cotton), Gel puncher or bore mouth 1 ml micro tip, Petridish.

V. PREPARATION OF REAGENTS

NOTE: The included buffers and reagents are optimized for use with this kit only. Substitution with other reagents may not give optimal results.

Assay Buffer: Prepare 1X **Assay buffer** by diluting it with distilled water (Add 20 ml distilled water to 5 ml 5X Assay buffer. The diluted buffer can be stored at 4°C for further use.

Preparation of Standards (serial dilutions)

Concentration of given Standard antigen is 2 mg/ml

1. Label four micro test tubes: 1:2, 1:4, 1:8, and 1:16.
2. Using a micropipette, add 50 µl of 1X Assay buffer to each tube.
3. With a fresh pipette tip, add 50 µl of Standard antigen to the tube labeled 1:2. Mix well.
4. With a fresh pipette tip, transfer 50 µl of the 1:2 dilutions to the tube labeled 1:4. Mix well.
5. With a fresh pipette tip, transfer 50 µl of the 1:4 dilutions to the tube labeled 1:8. Mix well.
6. With a fresh pipette tip, transfer 50 µl of the 1:8 dilutions to the tube labeled 1:16. Mix well.
7. There are now five antigen samples for the standard curve (see table).

Tube No.	Dilution	Concentration
1	Undiluted	2 mg/ml
2	1:2	1 mg/ml
3	1:4	0.5 mg/ml
4	1:8	0.25 mg/ml
5	1:16	0.125 mg/ml

VI. PROTOCOL

Preparation of Agarose Gel

1. Prepare 17 ml of 1.0% agarose (0.17 g) in 1X Assay buffer by heating slowly till agarose dissolves completely. Take care not to froth the solution.
2. Allow the molten agarose to cool to approx 55°C. Save approximately 2 ml of molten agarose solution for sealing the wells.
3. Add 450 µl of Antiserum to 15 ml of agarose solution. Mix by gentle swirling for uniform distribution of antibody.
4. Pour agarose solution containing the antiserum onto a clean Petridish and allow it to solidify for 15-20 minutes.
5. After solidification, the gel will appear slightly opaque.
6. Now punch the wells using gel puncher or with back of 1 ml micropipette tip corresponding to the template given below. The distances between the wells are important. Try to follow the template as accurately as possible.
7. Seal the wells with 20 µl of molten agarose solution per well and ensure that the distribution is uniform.
8. Allow them to solidify for 15-20 minutes.

Loading of sample

- a) Before loading label the wells on the bottom of the plate by marker as 1, 2, 3, 4, 5 and U in the centre of the plate as unknown sample.
- b) In well # 1, load 30 µl of the undiluted standard sample.
- c) In well # 2, load 30 µl of the 1:2 standard antigen dilution.
- d) In well # 3, load 30 µl of the 1:4 standard antigen dilution.
- e) In well # 4, load 30 µl of the 1:8 standard antigen dilution.
- f) In well # 5, load 30 µl of the 1:16 standard antigen dilution.
- g) Load 30 µl of test antigen in the center well (U).
- h) Label the cover of the Petridish. Place the dish (do not invert) inside the moist chamber (box containing wet cotton) and incubate at 37°C for overnight or at room temperature for 24 to 48 h.

Reading the results

9. The precipitin rings will be visible in 24 to 48 hours. Carefully hold a plate up so that the overhead room lights shine through it. You should be able to see opaque circles around each well where antigen and antibody have precipitated.
10. With a ruler, measure the diameter (through the centers of the wells) of the precipitin ring in millimeters. Note down your observations.
11. Plot a graph of diameter of ring (on Y-axis) versus concentration of antigen (on X-axis) on a semi-log graph sheet. Calculate the value of the unknown antigen concentration from the graph.

VII. TROUBLESHOOTING

Problem	Probable Cause	Suggestion
No precipitin line	Less incubation	Increase the incubation time.
No precipitin line	Degradation of antibody	Always add antibody at 55°C to the agarose gel.

VIII. REFERENCES

1. Mancini G, Carbonara AO and Heremans JF. *Immunochemistry*; 2:235-254 (1965)
2. B.T. Dumas, W. Watson and H.G. Biggs, *Clin. Chim. Acta*, 31:87-96 (1971).

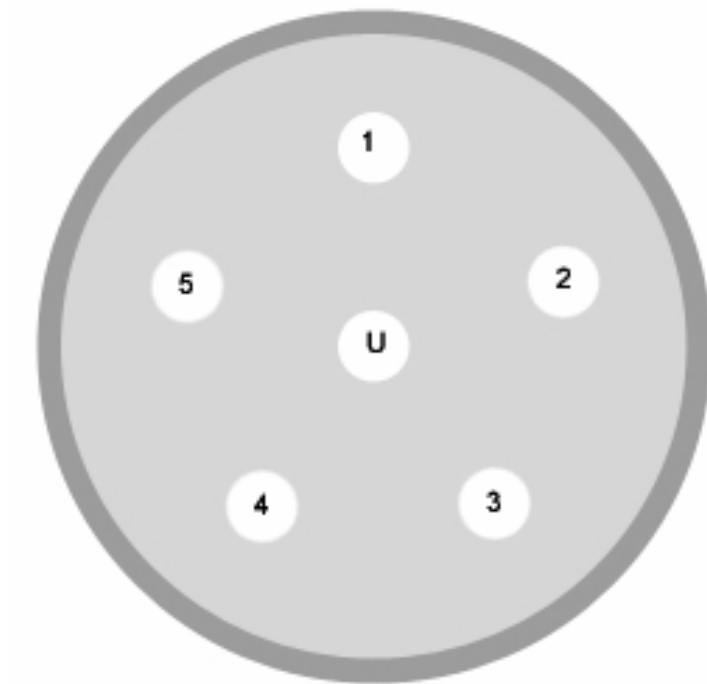


Figure 1. Template for Single Radial Immunodiffusion Assay