PermaRID® Radial Immunodiffusion Kit Catalog No. K10000

QED Immuodiffusion Kits provide *permanent* records of original immunodiffusion gels suitable for inserting into laboratory notebooks or manufacturing documents. This unique feature makes it possible to compare results over time and monitor consistency of antibody products. **PermaRID® Radial Immunodiffusion Kit** is designed for measuring antibody concentrations ≥50 µg/ml in murine ascites fluids, culture supernatants, or preparations of purified antibody.

Materials

Provided

1. Agarose gel containing polyclonal anti-mouse IgG/IgA/IgM
2. Purified Mouse IgG standards (1000, 500, 250, 62.5µg/ml concentrations)
3. Dilution buffer
4. Blotting paper

Required but not provided

1. Flat surface capable of gentle heating (temperature adjustable hot plate, slide warmer, etc...).
2. Distilled water
3. Micropipettors (20-1000µl)
4. Moisture proof container for incubation and washing
5. PBS-Phosphate (50mM) Buffered Saline (150mM NaCl), pH 7.4

Procedure

1. Make two dilutions of each antibody sample in dilution buffer (typically 1:20 and 1:50 for ascites and purified antibody preparations; 1:1 and 1:2 for culture supernatant).
2. Add approximately 20µl of the first standard to the first well (well should be filled completely). Continue adding 20 ul of each standard to the next three wells until the entire standard range has been added to the gel (see attached diagram).
3. Add 20µl of diluted antibody samples to the appropriate sample wells 1a through 13b (see attached diagram).
4. Store gel in a moist chamber at room temperature for 48 hours.
5. Wash the gel with PBS for 24 hours while shaking gently.
6. Place gel on a warming surface, and cover with blotting paper.
7. Flood blotting paper with distilled water and apply gentle heat.
8. When the gel and paper have dried, remove and discard paper.
9. Measure the diameter of the precipitin rings from the standards and samples if rings are hard to see put on dark surface or see attached stain and destain recipe. Record the data.
10. Plot the diameter of the standard rings against concentration.
11. Use linear regression to generate the standard curve.
12. Calculate x-intercept of the antibody samples from the precipitin ring diameter for each antibody sample.
13. Multiply the resultant concentration by the dilution factor of the antibody sample to determine Ig concentration per milliliter for the antibody.
14. Determine the average concentration for each antibody from the two dilutions. Record the data.
15. Dried PermaRID® gels may be stored indefinitely with conventional laboratory records.

Note: Quantification should be based on only those samples that have ring diameters that fall within the range of the standards for determining the antibody concentration. If both antibody dilutions fall out of the range of the standards, it may be necessary to repeat the RID at different dilutions.

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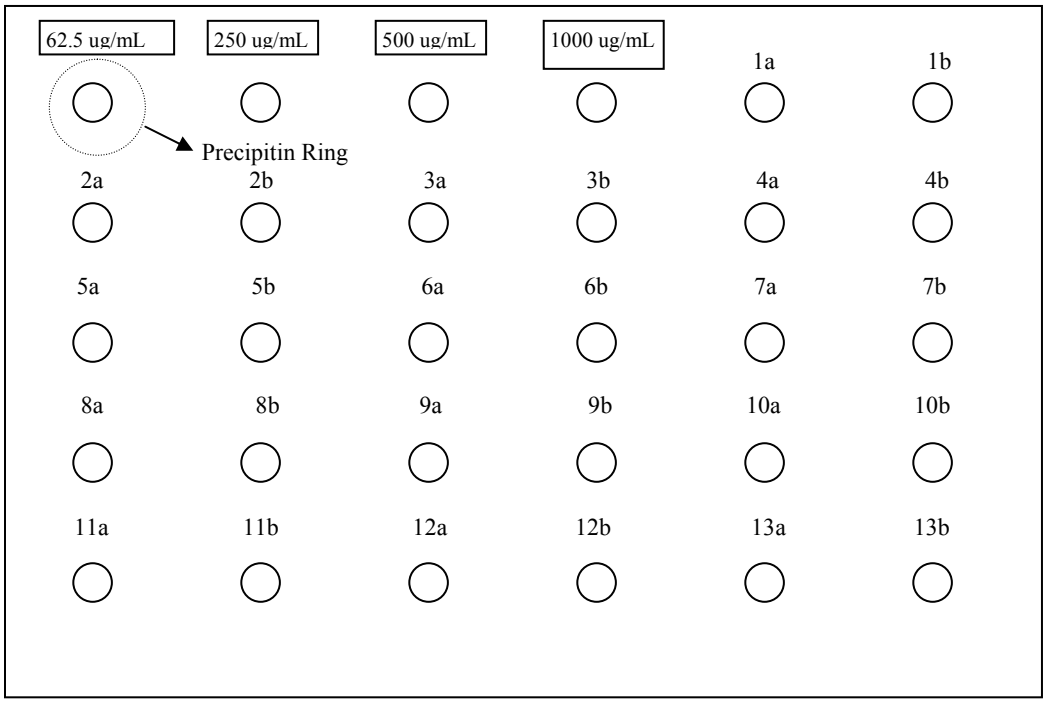

Description	Amount per liter	X	Batch Size (L)	=	Total Volume Required
Amido Black 10B	0.5g	X		=	ml
Methanol	450 ml	X		=	ml
Glacial Acetic Acid	100 mL	X		=	ml
Distilled Water	400 mL	X		=	mL

Submerse dried gel in stain for 10 min remoove and destain for 15 min let dry before reading

Description	Volume per liter	X	Batch Size (L)	=	Total Volume Required
Glacial Acetic Acid	100 ml	X		=	ml
Methanol	500 ml	X		=	ml
Distilled Water	400 mL	X		=	mL