QuantiType® Radial Immunodiffusion and Antibody Subtyping Kit

Catalog Nos. K15010, K15020, K15030, K15040, K15050

QED Immuodiffusion Kits provide *permanent* records of original immunodiffusion gel 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. **QuantiType® Radial Immunodiffusion and Antibody Subtyping Kits** are designed for simultaneous determination of antibody concentrations and subclasses in murine ascites fluids, culture supernatants, or preparations of purified antibody.

Materials

Provided

1. 1% Agarose gels containing subtype specific antiserum to mouse immunoglobulins.
2. Purified immunoglobulin controls at four different concentrations (.125, .25, .5, 1.0 mg/ml).
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 (20-1000 ul)
4. Moisture proof container for incubation and washing
5. PBS - Phosphate (50mM) buffered saline (150 mM NaCl), pH 7.4

Procedure

1. Make dilutions of each antibody sample in dilution buffer (typically 1:10, 1:50, 1:100 for ascites and purified antibody preparations, 1:2 or neat for culture supernatant).
2. Add approximately 20 ul of standard to duplicate wells (well should be filled completely). Continue adding standards to different wells until the entire standard range has been added to the gel (see attached diagram).
3. Add diluted antibody samples to sample wells.
4. Store in moist chamber at room temperature for 48 hours.
5. Wash the gel in PBS for 24 hours.
6. Place gel on a warming surface, and cover with blotting paper.
7. Flood blotting paper with distilled water, then 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. Dried QuantiType® gels may be stored indefinitely with conventional laboratory records.

Note: Quantitation should be based on only those samples which have ring diameters that fall within the range of the standards for determining the antibody concentration.

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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

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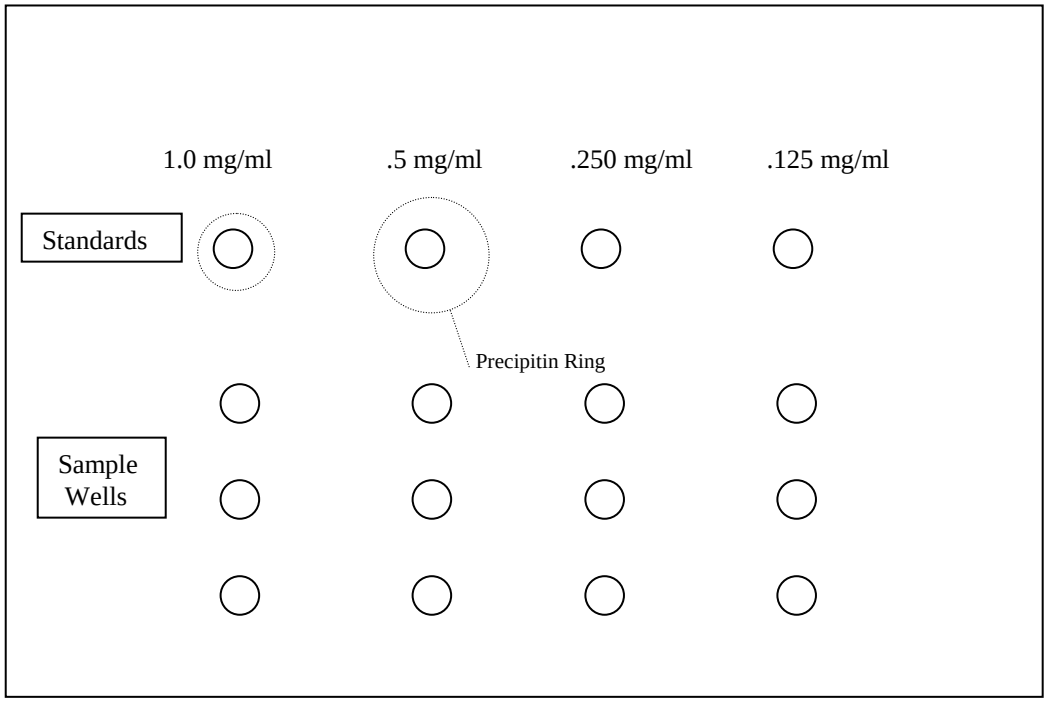
Recommended set-up of QuantiType® Gels:



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