QED PermaType® Antibody Subtyping Kit Catalog No. K20000

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. **QED PermaType® Antibody Subtyping Kit** is designed for determining antibody subclasses in murine ascites fluids, culture supernatants, or preparations of purified antibody.

Materials Provided

1. Antiserum to mouse immunoglobulin subtypes (Anti- IgA, IgG1, IgG2a, IgG2b, IgG3, IgM, kappa light chains); 200 ul containing 0.1% (v/v) sodium azide as preservative. Keep refrigerated when not in use.
2. Purified Immunoglobulins (IgA, IgG1, IgG2a, IgG2b, IgG3, IgM, kappa light chain); 75 ul containing 0.1% (v/v) sodium azide as preservative. Keep refrigerated when not in use.
3. Agarose gels sufficient for testing 25 antibody samples.
4. Dilution buffer
5. Blotting paper

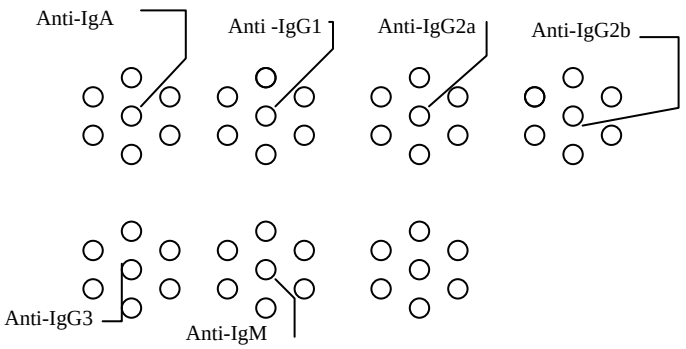
Materials Required but not provided

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

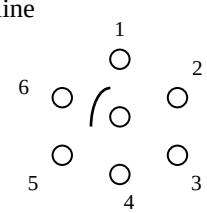
Procedure

1. Dilute samples in dilution buffer (ascites 1:200, culture supernatant 1:1, purified antibody at 10 µg/ml).
2. Add approximately 20 µl of diluted samples to each well surrounding the central well of a rosette pattern in gels. Use one of these wells for the antibody standard that will correspond to the anti-subtype antiserum used for each rosette pattern. Fill well to the top - do not overfill. There are eight different anti-subtype antiserum, so each sample should be placed in one well of eight rosette patterns. Record the well position of each sample.
3. Add approximately 20 µl of each anti-subtype antiserum to the central well of each rosette pattern.
4. Store in moist chamber at room temperature for 48 hours. A moist chamber can be a plastic container with a damp paper towel on which to rest the gels.
5. Wash gels for 24 hours by submerging gels in PBS and with a swirling motion, such as on a rocker or rotator platform.
6. Place gel on a warming surface, and cover with blotting paper.
7. Flood blotting paper with distilled water, and then apply gentle heat.
8. When the gel and paper have dried, remove and discard paper.
9. Examine the precipitin lines to determine subtype of the sample. The subtype of the sample corresponds to the antiserum used in the central well that produces a precipitin line. if rings are hard to see put on dark surface or see attached stain and destain recipe. Record the data.
10. Dried PermaType® gels may be stored indefinitely with conventional laboratory records.

Example of Gel arrangement



Example of a precipitin line



α-IgG1 antiserum used.
Sample #6 is an IgG1

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 remove 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