# QED Bioscience Inc.

ADVANCED RESEARCH TECHNOLOGIES

**PermaRID™ Custom Immunodiffusion Kit** **Catalog No. K11000**

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.
**PermaRID™ Custom Immunodiffusion Kit** is designed for laboratories that wish to design their own RID systems. Simply add the anti-immunoglobulin reagent of choice to the agarose provided and create the RID system that you need.

## Materials

**Provided**

1. 2% Agarose gel
2. Gel support substrate
3. Dilution buffer: One vial with no indicator dye (Buffer A), One vial containing indicator dye (Buffer B)
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
6. Tool to punch wells in the gel
7. Vacuum system to remove agarose from well

## Procedure

1. Melt 2% agarose in boiling water bath or microwave. Keep in 56°C water bath until ready to use. This procedure requires approximately 40ml of 1% agarose for a 110 x 125 mm sheet of Gel support substrate.
2. Pre-warm Buffer A in 56$^{o}$ C water bath.
3. Add 0.5 ml of antiserum to 20 ml of Buffer A.
4. Dilute 20 ml of 2% agarose with 20 ml of Buffer A containing antiserum , and gently mix.
5. Gently pour agarose onto Gel support substrate. Any bubbles that are present can be removed while the agarose is still liquid by gently touching with the corner of a paper towel.
6. Allow gel to harden at room temperature. (Gels may be stored in a moist chamber at 4°C for up to one week)
7. Punch 4 mm holes according to template (see below)
8. Make dilution’s of known concentrations of antigen in Buffer B. A minimum of four standards should be generated (125, 250, 500, 750 ug/ml; additional concentrations can be used to expand the standard range)
9. Make dilutions of antigen samples in Buffer B.

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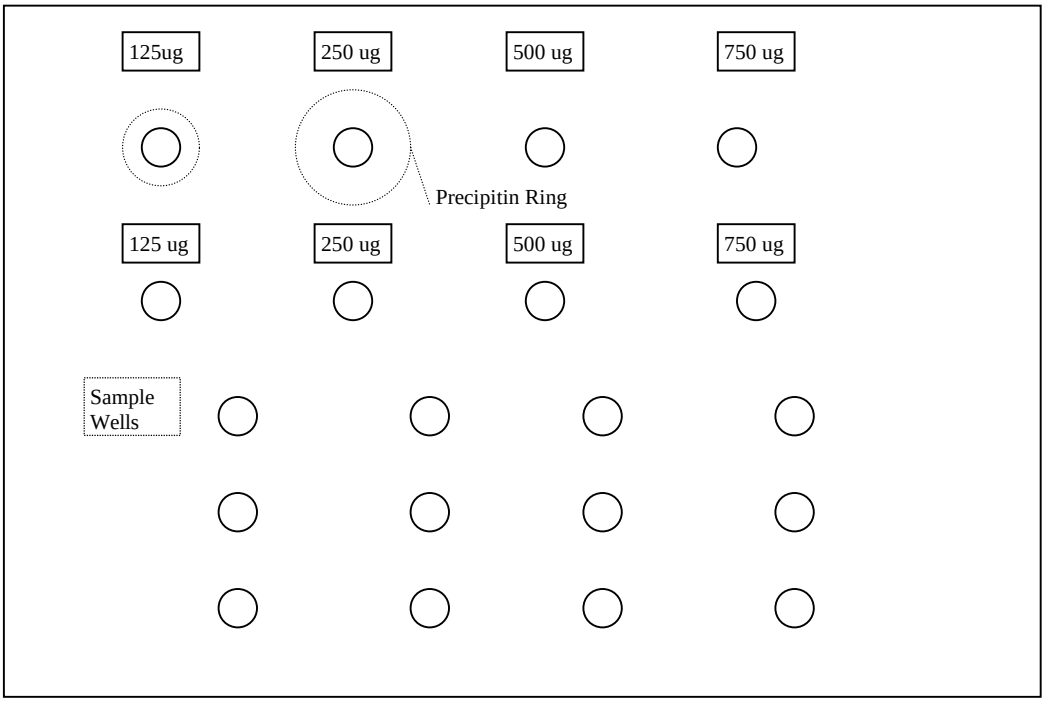
10. Add approximately 20 ul of standard to a well (well should be filled completely). Continue adding standards to different wells until the entire standard range has been added to the gel (see attached diagram).
11. Repeat step 10 for each antigen sample.
12. Store in moist chamber at room temperature for 48 hours.
13. Wash the gel in PBS for 24 hours.
14. Place gel on a warming surface, and cover with blotting paper.
15. Flood blotting paper with distilled water, then apply gentle heat.
16. When the gel and paper have dried, remove and discard paper.
17. Measure the diameter of the precipitin rings from the standards and samples. Record the data.
18. Plot the diameter of the standard rings against concentration.
19. Use linear regression to generate the standard curve.
20. Calculate x-intercept of the antibody or antigen samples from the precipitin ring diameter for each sample.
21. Multiply the resultant concentration by the dilution factor of the sample to determine the concentration for the antibody or antigen.

    **Note: use only those samples which have ring diameters that fall within the range of the standards for determining the antibody concentration.**

Dried **PermaRID™** gels may be stored indefinitely with conventional laboratory records.





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