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ProductInformation

AGAROSE
High EEO

Product Number **A 5304**
Store at Room Temperature

Procedure

Preparation and Use of Counter Immunoelectrophoresis Gels

1. Dissolve one vial Barbital Buffer (Product No. B 6632) in one liter deionized water.

2. Prepare 30 ml of 1% agarose solution (sufficient for two 85 x 100 mm gels) by mixing 0.3 g agarose (Product No. A 5304) in 29 ml Barbital Buffer (Product No. B 6632) and heat in a boiling water bath until completely dissolved.

3. Pour solution onto hydrophilic side of a level, well supported Electrophoresis Film for Agarose Gels (Product No. E 0264) sheet (85 x 100 mm) from the center outward forming an even layer 1-1.5 mm thick.

4. Allow the gels to harden for one hour at 4 °C or overnight at room temperature.

5. Punch centrally positioned 2 mm diameter wells, 5 mm apart in a line parallel with gel's long side. Position the wells in a cathode-anode alignment.

6. Load 5-10 µl of a 1% antigen solution in the cathode well and 5-10 µl of an appropriate antibody solution in the anode well.

7. Electrophorese the gel at 5 volts/cm (8.5 cm width) for approximately 1 hour using the above Barbital Buffer as electrode solution. A precipitin line between the wells should form.

8. To enhance visualization of the precipitin band, elute the unreacted protein, then dry and stain the gel with an appropriate solution from the Electrophoresis section of the Sigma catalog.

References

Bryan J. Culliford, Nature, **201,** 1092 (1964).

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