

Tissue culture embedment in culture dishes

Solutions:

1. Cacodylate buffer: 0.1 M NaCl in 0.05 M Cacodylate; pH 7.5
2. Fixative: 2% or 3% glutaraldehyde in cacodylate buffer
3. Phosphate buffered saline (pbs); pH 7.5
4. Osmium tetroxide: 1% in pbs

Protocol:

1. Remove dishes from incubator and pour off media; rinse the dish with pbs.
2. Add fixative to the dish and allow to sit at room temperature for 30 minutes.
3. Rinse dish 2 times with pbs and replace second rinse with osmium tetroxide. Leave 30 minutes.
4. Rinse dish 1 time with pbs and 2 times with dH₂O.
5. Wash with 30, 50, and 70% hexylene glycol for 20 minutes for each washing. Dishes can be refrigerated until ready to embed.
6. Wash with 100% hexylene glycol 3 times for 10 minutes apiece.
7. Combine 1 part epoxy resin to 2 parts hexylene glycol and add to dish for 2 hours.
8. Combine 2 parts epoxy resin to 1 part hexylene glycol and add to dish for 2 hours.
9. Place pure epoxy resin in dish and place on rotor for 5-7 hours.
10. Embed in pure resin and bake for 12-14 hours and then break dish away from sample. Note: use only a thin layer (about 2-3 mm) of resin, to facilitate separation from dish surface.
11. Bake for an additional 24 hours. Sample can be glued to blocks for sectioning.