

Inverted Embedding Capsule Method for Cultured Cells

1. Rinse cell monolayer with phosphate buffered saline (PBS) (1×, 3 min).
2. Incubate cells with PBS containing 2.5% glutaraldehyde and 2% paraformaldehyde for 1h at room temperature (RT).
3. Remove fixative then add PBS (3×, 10 min).
4. Incubate the cells with 25% ethanol (1×, 10 min), 50% ethanol containing 1% uranyl acetate (for *en bloc* staining; 1×, 60 min, in the dark), 70% ethanol (1×, 10 min), 95% ethanol (3×, 10 min), then 100% ethanol (3×, 10 min).
5. Incubated the cell monolayer with epoxy resin (3×, 60 min).
6. Remove the epoxy resin then invert embedding capsules full of resin over the relevant areas of the monolayer the polymerize in an oven at 37°C for 12h then 70°C for 48h.
7. Pop off the embedding capsules and underlying cells from the bottom of the petri dish. The cells will be near the surface of the epoxy resin block.
8. Section resin blocks containing cells of interest with an ultramicrotome.
9. Collect sections on formvar- and carbon-coated 200 mesh copper TEM grids.
10. Stain sections with 1% filtered uranyl acetate (5 min) then Reynold's lead citrate (2 min).
11. Image the grids.

Epoxy Resin	
Embed 812	10.0 g
DDSA	4.5 g
NMA	6.0 g
DMP-30	0.35 ml