

## Routine Embedding Method

**Warning:** Uranyl acetate is toxic and radioactive. A discussion of the safe handling of this material can be found [here](#).

### *Materials*

#### *Formaldehyde-Glutaraldehyde-Picric-Acid Fixative*

Paraformaldehyde (Electron Microscopy Sciences (EMS), CAT #19208)  
Sodium Hydroxide (1N, EMS, CAT #21170-01)  
Picric Acid (EMS, CAT #19550)  
0.2M Sodium Cacodylate buffer (EMS, CAT #11650)  
Calcium Chloride, Dihydrate (EMS, CAT #12340)  
25% Glutaraldehyde, EM grade (EMS, CAT #16210)

#### *Uranyl Acetate Stain for Electron Microscopy*

0.2M Tris Maleate Buffer, pH 5.15 (EMS, CAT # 11740)  
Uranyl Acetate (EMS, CAT #22400)

#### *Osmium Tetroxide Post-fix*

2% Osmium Tetroxide Solution (EMS, CAT #19152)  
0.2M Sodium Cacodylate buffer (EMS, CAT #11650)

#### *Dehydration*

Ethanol (200 Proof, USP/ACS, Pharmco, CAT #111000200)  
Propylene Oxide (EMS, CAT #16210)

#### *Embedding Media*

Poly/Bed<sup>®</sup> 812 (Polysciences Inc., CAT #08791)  
Nadic Methyl Anhydride (Polysciences Inc., CAT #00886)  
Dodecenylsuccinic Anhydride (Polysciences Inc., CAT #00563)  
DMP-30 (Polysciences Inc., CAT #00553)

**Note:** the above reagents are now available as a kit- Poly/Bed<sup>®</sup> 812 (Luft formulations) Embedding Kit/DMP-30 (Polysciences Inc., CAT #08792)

Embedding mold  
Vacuum Oven  
Disposable pipettes  
Eppendorf tubes  
Scintillation vial

### *Procedure*

**Note:** Tissue culture cells are fixed *in situ* while adhered to the culture dish and later lifted off with propylene oxide (see step 8 below) to maintain cell structure (trypsinizing cells causes them to round up and lose their original shape).

1. Fix whole tissue or cultured cells for at least 1 hour or 45 minutes, respectively, using the “2/3FGP” fixative procedure described in detail [here](#).
2. Post-fix tissues and cells with osmium tetroxide as described in detail [here](#), ensuring that the tissue/cells are barely submerged.
3. Rinse with 0.2M sodium cacodylate buffer 2-3 times quickly.
4. Wash 3 times with 0.2M tris maleate buffer.
5. Soak in 1% uranyl acetate in 0.2M tris maleate buffer for 1 hr (tissue) or 20-30 min (cells).
6. Wash 3 times in 0.2M tris maleate buffer.
7. Dehydrate the tissue/cells by soaking them in a succession of increasing concentrations of ethanol as follows:

50% cold ethanol (dehydration of cells is an exothermic reaction and will give off heat)

80% cold ethanol

95% cold ethanol

Soak in each solution for 1 min (tissue) or 50sec (cells)

100% room temperature ethanol. Soak for 1h (tissue) or 20-30 minutes (cells). Repeat with fresh ethanol twice more.

**Note:** Steps 5-7 can be omitted if the sample is not to be used for electron microscopy

8. For cultured cells- add sufficient propylene oxide to cover the cells in the culture dish. Cells should float off of the surface. Remove the cells immediately since the propylene oxide will continue to dissolve the dish. Transfer the cells to a centrifuge tube and soak the cells for 20 to 30 minutes. Spin the cells down in a centrifuge tube to a tight pellet. Resuspend the pellet in propylene oxide and repeat the 20 to 30 minute soak followed by centrifugation. For tissue- soak tissue for 1 hour in propylene oxide. Repeat twice with fresh propylene oxide.

9. Infiltrate the tissue or cell pellet for several hours to overnight (covered) in Poly/Bed<sup>®</sup> 812 epoxy containing hardener and catalyst (epoxy preparation and storage is described in detail [here](#)) and mixed with equal parts propylene oxide (“1/2 & 1/2”).

- The longer the better for infiltration.
- If leaving overnight, put in the refrigerator to prevent the epoxy from becoming too sticky.

The next day, let samples sit out in the hood to warm before embedding.

10. To embed, warm the 1/2 & 1/2 epoxy (to make it less viscous) in the 60° C vacuum oven for 10-15 min. Pour the warmed epoxy into a suitable mold. Let the poured epoxy sit in oven 5 minutes before embedding to let air bubbles rise. Immerse infiltrated tissue or cell pellet into the epoxy and let sit in a 60° C oven under vacuum until hardened, at least 8 hours.

- For larger pieces of tissue, dip first into a 100% epoxy mixture before embedding to let propylene oxide evaporate off.
- The evaporation of the propylene oxide produces bubbles in the mold.

**Adapted from:** Karnovsky, MJ (1965) A formaldehyde-glutaraldehyde fixative on high osmolarity for use in electron microscopy. *J Cell Biol* **27**, 137A -138A.