

Routine Morphology Protocol

1. Fix tissue (2% gluteraldehyde, + 3% paraformaldehyde, + 5% Sucrose) in Sodium Cacodylate buffer (pH7.4)
2. Wash 0.1M cacodylate buffer + 7.5% sucrose.
3. Post fix Palade's Osmium one hour on ice in light tight container.
Palade's Osmium
1% osmium
Acetate veronal
0.01N HCl
ddH₂O
4. Rinse 1X Kellenbergers UA. Then incubate 2-4 hours in Kellenberger's (overnight)
Acetate Veronal
HCl
Uranyl Acetate
H₂O
5. Quick rinse in cold 50% ETOH
6. Dehydrate with graded series of ethanol (75,90,95,100,100 100) 5 min each.
Place in a tube with 50% (100%) ETOH and 50% Propylene oxide 5 min.
Place in a tube with 100% Propylene oxide 5-10 min.
7. Place in a tube with 50%PO + 50% Epoxy resin. Let sit on rotator or under vacuum overnight.
8. Replace epon with fresh 2X, leave under vacuum.
9. Place tissue in mold and cure at 60^o overnight.
10. Start cutting.
11. Pick up sections on formvar coated grids, stain with UA (20 min) and Lead citrate (4-6min)
12. Scope.

Protocol taken from the W.M. Keck Microscopy Facility at The Whitehead Institute website