

GENERAL FIXATION AND EMBEDMENT PROCEDURE FOR BIOLOGICAL TISSUE.

1. Primary Fixation: 1-2% paraformaldehyde, 2-4% glutaraldehyde, in a suitable pH buffer (i.e. 6.8-7.2 pH 0.2 M Sodium Phosphate, 0.05 M Sodium Cacodylate, HEPES, PIPES, etc.). Fixation for 5-120 min. at room temp. (20-22 C), normal growth temp (37 C?) or on ice (0-4 C). [50 min. @ room temp]. Specimens should be cut as small as possible (0.5-1 mm cubes).
2. Rinse: 4 times @ 15-20 min. each with the above buffer without aldehyde fixatives. (Residual aldehydes will bind with OsO₄ in secondary fixation if used.)
3. Secondary Fixation: 1-2% Osmium tetroxide (OsO₄) in full to half strength buffer used above. Fixation for 2-6 hours at room temperature. (OsO₄ fixation generally not used if immunological staining procedures will be used.)
4. Rinse: 4 times @ 15-20 min. each with distilled water.
5. *En bloc* Staining (tertiary fixation): 0.05% Uranyl acetate in water for 8 - 18 hours, i.e. overnight.
6. Rinse: 4 times @ 15-20 min. each with distilled water.
7. Dehydration: Generally either absolute ethanol (200 proof) or glass distilled acetone is used.

% Solvent in water	Time	
25%	20-30 min	
50%	20-30 min	
75%	20-30 min	
95%	30-60 min	
100%	60+ min	
100%	60+ min	
100%	60+ min	(If samples are held at 100% for more than 8 hours an additional 100% step is recommended before next step.)

SEM

8. Critical Point Drying: Samples for SEM imaging should be critical point dried at this point.

TEM

9. Infiltration: Samples for TEM Embedment should be infiltrated with the embedding resin according to procedures recommended for that resin. In general for epoxy

resins, i.e. Spurr's, Spurtol, or Quetol 651 resins, the following infiltration schedule is suggested.

<u>Resin : Solvent</u>	<u>Time*</u>
1:3 (25%)	2-3 hours
2:2 (50%)	2-3 hours
3:1 (75%)	2-3 hours
100% resin	2-3 hours
100% resin	2-3 hours

* Any of these steps can be increased in duration to accommodate such necessities as sleeping. If the 100% steps are held for longer than 8 hours an additional 100% step may be needed.

10. Polymerization: Samples are placed into suitable molds with 100 % resin and appropriate paper labels written with either pencil or laser printed (Note: INK LABELS ARE NOT ACCEPTABLE AS THEY WILL BLEED DUE TO THE SOLVENT NATURE OF THE RESINS!). It is suggested that the embedding molds be partially pre-filled with a small quantity of resin (approx 1-2 mm) which is polymerized for 4-8 hours in order to raise the samples off the bottom of the molds and facilitate sectioning. Samples should be polymerized for 24-72+ hours at 62-70 C. (Oxygen exclusion, i.e. use of vacuum ovens, is not needed for epoxy resins).
11. Following polymerization specimen blocks can be stored for indefinitely (2,000 yrs or more) before ultrathin sectioning. Storage in a desiccator (24+ hours) prior to sectioning seems to facilitate ultrathin sectionability.
12. Ultrathin sectioning.
13. Post-sectioning staining.