

## Embedding for light microscopy - routine structure

1. Fix in 3% glutaraldehyde in 25 mM phosphate buffer, pH 7.2, at room temperature for 90 min or longer. If necessary, vacuum infiltrate first for a few min until material sinks.
2. Rinse in 25 mM phosphate buffer 4 times over approx 60 min.
3. Dehydrate in ethanol series:

Concentration of ethanol	Small tissues	Larger, denser tissues
25%	1-2 h	At least 8 h
50%	1-2 h	At least 8 h
70%	overnight at 4°C	At least 8 h
95%	2 h	At least 8 h
100%	1-2 h, repeat at least 3 times	At least 8 h, repeat 3 times

4. Infiltrate in LR White resin (medium grade):

Small tissues		Larger, denser tissues	
Concentration of resin	Regime	Concentration of resin	Regime
2:1 ethanol : LRW	8 h to overnight	10%	At least 24 h
1:2 ethanol : LRW	8 h to overnight	25%	At least 24 h
100% LRW	At least overnight, then repeat 3 times, at least 8 h each	50%	At least 24 h
		70%	At least 24 h
		90%	At least 24 h
		100%	At least 24 h, then repeat twice (4 h each)
		100%	Change every second day for a week

5. Flat embed in foil planchettes. Polymerise a thin layer of resin on the bottom of the planchettes before embedding (at 60°C under nitrogen gas). Alternatively, embed in rubber moulds or gelatine capsules. In each case, a thin layer can be polymerized first to facilitate positioning of tissue in mould.
6. Polymerise in vacuum oven at 60°C for about 90 minutes under nitrogen gas (polymerisation is complete when resin is hard), adjust flow rate to give 250-300 kPa on the nitrogen tank gauge.

Waste LRW and LRW-solvent mixtures go into glass jar in fume hood.

Waste LRW-contaminated pipettes go into plastic container in fume hood.

**Notes:**

- i. Larger and denser tissues need longer in every stage. For example, cereal grains can be fixed overnight or longer, need long vacuum infiltration of fixative, need at least 8 hours in each dehydration step (preferably longer), and need slow infiltration with resin – smaller increases in resin concentration and longer at each stage – at least a day (24 h) in each. Very hard/dense tissues may need several days to several weeks for resin infiltration.
- ii. Larger tissues (as above) may need to be vacuum infiltrated in resin to remove any remaining solvent.
- iii. Impermeable tissues need slower and longer infiltration or the tissues will shrink and be deformed – impermeable walls allow solvent (EtOH) to diffuse out much more rapidly than the resin monomers can diffuse in, hence cell collapse. For example, *Arabidopsis* roots show cell collapse if the resin concentration is increased too rapidly – commonly seen in GUS-stained tissues if not careful.
- iv. Hard tissues such as dry grains may also need to be embedded in harder resin – hard grade rather than medium grade.