

Standard Fixation for Biological Material for TEM

Fixation Schedule

Fix:	3.5% Glutaraldehyde in 0.1 M PO ₄ buffer	1/2 to 2 hours on ice
Rinse:	2 times in 0.1 M PO ₄ buffer	15 minutes each
Post-fix:	2% OsO ₄ + 0.2 M PO ₄ buffer (1:1)	1 1/2 to 2 hours or overnight on ice
Rinse:	0.1 M PO ₄ buffer	15 minutes
Dehydrate:	EtOH	15 minutes minimum each step
	25%	
	40%	
	60%	
	80%	
	95%	
	100%	
	** 100% (1 hour)	
	100% (store overnight refrigerated)	

**Processing may be arrested at this point until continuation is convenient.

Transition to resin

EtOH : Acetone (2:1)	15 minutes
EtOH : Acetone (1:2)	15 minutes
100% Acetone	15-30 minutes
100% Acetone	15-30 minutes
Acetone : Resin (3:1)	2-3 hours
Acetone : Resin (1:1)	2-3 hours
100% Resin	overnight on shaker

Embedding

Fill molds with fresh resin

Transfer samples to molds

Dessicate for 2-3 hours

Place molds in oven for at least 8 hours