

Conventional TEM sample preparation of Tissue

Day 0 by Manual				
Steps		Time	Temp	Check
Pre-fixation	3% Glutaraldehyde + 3% Paraformaldehyde	Overnight	4 °C	<input type="checkbox"/>
	in 0.1M Cacodylate Buffer, pH 7.2			

Day 1 by Manual				
Washing	0.1M CB	15 m x 3	RT	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Post-fixation	1.5% K ₄ Fe(CN) ₆ + 2% OsO ₄ in 0.1M CB	1 h x 1	ICE	<input type="checkbox"/>
	0.3 ml 10% potassium ferrocyanide K ₄ Fe(CN) ₆ , 1% Final			
	0.5 ml 4% OsO ₄ , 1% Final			
	0.5 ml 0.4M CB, 0.1M Final			
	0.2 ml D.W.			
	2 ml Total			
Washing	0.1M CB	3 m x 3	RT	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Washing	0.1M Na ₂ ⁺ Acetate Buffer, pH 5.2	3 m x 3	RT	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
en bloc Stain	2% UA in 0.1M Na ₂ ⁺ Acetate, pH 5.2	1 h x 1	RT	<input type="checkbox"/>

Day 1 by Automated Tissue Processor (Program #2)				
				Station #
Washing	0.1M Na ₂ ⁺ Acetate Buffer, pH 5.2	5 m x 2	RT	1, 2
Washing	MQW	5 m x 1	RT	3
Dehydration	30% EtOH	15 m x 1	4 °C	4
	50% EtOH	15 m x 1	4 °C	5
	70% EtOH	15 m x 1	4 °C	6
	80% EtOH	15 m x 1	4 °C	7
	90% EtOH	15 m x 1	4 °C	8
	95% EtOH	15 m x 1	4 °C	9
	100% EtOH (I)	15 m x 1	RT	10
	100% EtOH (II)	15 m x 1	RT	11
	Acetone	15 m x 1	RT	12
Infiltration	Acetone : Spurr's Resin = 2 : 1	1 h x 1	RT	13
	Acetone : Spurr's Resin = 1 : 1	2 h x 1	RT	14
	Acetone : Spurr's Resin = 1 : 2	16 h x 1	RT	15
	Absolute Spurr's Resin	24 h	RT	16

Day 2 by Manual				
Embedding	Beam capsule size or flat embedding mold & labeling	30 m	RT	<input type="checkbox"/>
Polymerization	Oven	24 h	65 °C	<input type="checkbox"/>

Day 3 by Manual				
Sectioning	Ultramicrotome sectioning			<input type="checkbox"/>
Post-staining	4% Uranyl Acetate & Reynolds' Lead Citrate			<input type="checkbox"/>