

Conventional TEM sample preparation of Cell Monolayer

Day 0 by Manual

Steps		Time	Temp	Check
Pre-fixation	2.5% Glutaraldehyde in 0.1M Cacodylate Buffer, pH 7.2	Overnight	4 °C	<input type="checkbox"/>

Day 1 by Manual

Washing	0.1M CB	5 m x 3	RT	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Post-fixation	1% K ₄ Fe(CN) ₆ + 1% OsO ₄ in 0.1M CB	15 m x 1	ICE	<input type="checkbox"/>
	0.2 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			
	0.8 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	2 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	15 m x 1	RT	<input type="checkbox"/>

Day 1 by Automated Tissue Processor (Program #1)

				Station #
Washing	0.1M Na ²⁺ Acetate Buffer, pH 5.2	3 m x 3	RT	1, 2, 3
Washing	MQW	3 m x 1	RT	4
Dehydration	10% EtOH, ice-cold	5 m x 1	4 °C	5
	30% EtOH, ice-cold	5 m x 1	4 °C	6
	50% EtOH, ice-cold	5 m x 1	4 °C	7
	70% EtOH, ice-cold	5 m x 1	4 °C	8
	80% EtOH, ice-cold	5 m x 1	4 °C	9
	90% EtOH, ice-cold	5 m x 1	4 °C	10
	95% EtOH, ice-cold	5 m x 1	4 °C	11
	100% EtOH (I)	5 m x 1	RT	12
	100% EtOH (II)	5 m x 1	RT	13
Infiltration	100% EtOH : Spurr's Resin = 2 : 1	30 m x 1	RT	14
	100% EtOH : Spurr's Resin = 1 : 1	1 h x 1	RT	15
	100% EtOH : Spurr's Resin = 1 : 2	4 h x 1	RT	16
	Absolute Spurr's Resin	Overnight	RT	17

Day 2 by Manual

Embedding	Beam capsule size 3 & labeling	15 m	RT	<input type="checkbox"/>
Polymerization	Beam capsule only	6 h	65 °C	<input type="checkbox"/>
	Fill absolute Spurr's resin into the beam capsule	Overnight	65 °C	<input type="checkbox"/>

Day 3 by Manual

Sectioning	Ultramicrotome sectioning			<input type="checkbox"/>
Post-staining	2% Uranyl Acetate & Reynolds' Lead Citrate			<input type="checkbox"/>