TEM Tissue Processing

Prior to starting an Electron Microscopy Core project, investigator must meet with Senior Scientist Anza Darehshouri to discuss project goals, appropriate processing protocols, microscopes and request fixation solutions for preserving tissues.

The facility is unable to accept tissue which has not been fixed. Please mince tissue to small size (approx. 1 mm square or smaller). Ensure that tissue does not dry out during this time period. Artifacts will be evident in the TEM if tissue is allowed to dry.

Routine TEM tissue processing usually takes three days from process start date. Additionally, ultramicrotomy and grid staining must also be done before the specimen is ready for the scope. Thus, the minimum turn around time for standard processing is no less than one week from date of submission.

Day 1

1.	Primary Fixation	2.5% Glutaraldehyde in 0.1M Cacodylate buffer, pH 7.4 @RT for 1.5 hours, or overnight 4^0C
2.	Rinse 5X	0.1M Cacodylate buffer pH7.4 @ RT 5-8 minutes each
3.	Secondary Fixation 1-2X	1% Osmium Tetroxide with 0.8% $K_3Fe(CN_6)$ in cacodylate buffer for 1.5 hours RT in dark
4.	Rinse 5X	Double distilled water @RT 5-8 minutes each (last wash overnight @4^0C)

Day2

1.	Pre-Stain	4% Uranyl Acetate in 50% ETOH @ RT 2 hours in dark	
2.	Dehydration	50% Ethanol in DH_2O	1X @RT for 8 minutes
		70% Ethanol in DH_2O	2X @RT for 8 minutes
		85% Ethanol in DH_2O	1X @RT for 8 minutes
		95% Ethanol in DH_2O	2X @RT for 8 minutes
		100% in DH_2O	4X @RT for 8 minutes (Open new 100% ETOH bottle if more than 1 week old)
3.	Transition	Propylene Oxide (PO)	1X @RT for10 minutes
4.	Infiltration	PO:EPON (2:1)	1X @RT for 2+ hours on rotator
		PO:EPON (1:2)	1X @RT for 2+ hours on rotator then overnight

Day3

1.	Infiltration	100% EPON	2X @RT for 2+ hours on rotator
2.	Embed	100% EPON	Add paper label with sample ID to embedding mold
3.	Polymerization	Place embedding molds in 70^0C oven overnight	

Date:
Researcher:
P.I.:
Specimen: