

Nils Hasselmo Hall/ Char Fac TEM Tissue Processing Check List

	Date: _____	Time Needed / Step:	StartTime: or ✓ when step is completed
Day 1:	Tissue/ User: _____		
1° Fix: 2 hr	1.	Glut fix	1 hr minimum- <i>may hold in refrig at this point.</i>
	Held in refrig? Start date: _____ End Date: _____		
Day 1 or 2:	2.	First buffer rinse	3 x 5 min
	Start resin preparation. > At final, use vacuum to release bubbles.		
Postfix: 1 hr	3.	Post fix at room temp or ice, cover samples.	45 min
	5.	Rinse buffer	2 changes x 5 min
Dehydration: 2 hr	6.	Rinse dH2O	1 change x 5 min
	7.	50% ETOH	10 min
	8.	70% ETOH	10 min
	9.	70% ETOH	10 min- <i>may hold in refrig at this point.</i>
	Optional: en bloc staining: _____ Start: _____ End: _____		
Labor Time: 4.0 hr.	10.	80% ETOH	10 min
	11.	95% ETOH	10 min
	12.	95% ETOH	10 min
	13.	ABSOL ETOH	10 min
	14.	ABOSL ETOH	10 min
	15.	Propylene Oxide	10 min
	16.	Propylene Oxide	10 min
Time: 3.5 hr.	If small batch of tissue, split resin into half (12.5ml) and put DMP-30 (0.25ml) into one half. Mix and use. Wrap 2 nd half tightly and put into freezer for tomorrow's processing.		
Infiltrate: 1-2 hr.	17.	33% Resin: 67% Propylene Oxide (1:2)	60 min-120 min on rotator w/caps off.
T. Time: 5+ hr.	Prepare labels and molds		
Overnight 50% resin	18.	50% Resin: 50% Propylene Oxide (1:1)	overnight on rotator
T. Time: 5+ hr.	19.	100% Resin	8 hr-to-overnight on rotator in hood, vials uncovered.
100% resin Infiltrate 8 hr	Day 2 or 3:		
	Start resin preparation. > At final, use vacuum to release bubbles.		
T. Time: 6+ hr.	20.	Fresh 100% resin-for embedding	Moulds sit on bench for 1 hr before 40C oven.
Orient tissue; slight vacuum	Days 3-5:		

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21.	40C oven for 24 hr; 60C oven for 48 hr	Cool in mould for 1 hr before removing.	
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T. Time: 7.5+ hr.