

NHH/ CharFac TEM Tissue Processing Check List for Cell Monolayers:

_____		Time Needed/ Step:	Start Time: and ✓ when step is completed
Tissue/ User: _____			
1.	Glutaraldehyde fixation	1 hr minimum- may hold in refrig at this point.	
2.	1 st Rinse buffer	3 x 1 min	1. 2. 3.
Start resin preparation. > At final, use vacuum to release bubbles.			
3.	2 nd Rinse buffer	1x 1 min	1.
4.	Post fix at room temp or ice. Cover samples.	45 min	
5.	Rinse buffer	3 x 1 min	1. 2. 3.
6.	35% ETOH	2 x 2 min	1. 2.
7.	50% ETOH	2 x 2 min	1. 2.
8.	70% ETOH	2 x 3 min	1. 2.
9.	80% ETOH	2 x 3 min	1. 2.
	Cells cannot become exposed to air:		
10.	95% ETOH	2 x 2 min	1. 2.
11.	95% ETOH	2 x 3 min	1. 2.
12.	100% ETOH	2 x 2 min	1. 2.
13.	100 % ETOH	2 x 3 min	1. 2.
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 next day processing.			
14.	1:3 Epon mix (1 part resin to 3 parts 100% ETOH)	10 min on rotator uncovered. Speed of 2.0.	
15.	1:2 Epon mix (1 part resin to 2 parts 100% ETOH)	15 min on rotator uncovered.	
16.	1 st 1:1 Epon mix (1 part resin to 1 part 100% ETOH)	15 min on rotator uncovered.	
17.	2 nd 1:1 Epon mix	15 min on rotator uncovered.	
18.	100% Resin	20 min on rotator uncovered.	
19.	100% Resin	20 min on rotator	

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		uncovered.	
20.	100% Resin	20 min on rotator uncovered.	
21.	100% Resin	20 min on rotator uncovered.	
22.	Put in 60°C oven	48 hours	
23.	Crack petri dishes and put in 40 or 60°C oven depending on resin		