

Processing and Embedding of Cell Suspension

Steps	Reagent	Rinses and Time	Done
Fixation			
1. pellet cells, wash with 0.1M Cacodylate buffer	0.1M Cacodylate Buffer	3 x 5 minutes	1. 2. 3.
2. replace buffer with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2)	2.5% glutaraldehyde in 0.1M Cacodylate buffer	40 minutes -room temperature	1.
3. wash cells with 0.1M cacodylate buffer	0.1M cacodylate buffer	3 x 5 minutes	1. 2. 3.
Post-Fixation			
4. postfix cells in 1% osmium tetroxide in 0.1M cacodylate buffer	1% osmium in 0.1M cacodylate buffer	30 minutes at room temperature in the dark	1.
5. wash in cacodylate buffer (pH 7.3)	0.1M cacodylate buffer	1x rinse 2 x 5 minutes	1. 2. 3.
Dehydration			
6.	30% ethanol	2 x 5 minutes	1. 2.
7.	50% ethanol	2 x 5 minutes	1. 2.
8.	70% ethanol	1 x 5 minutes	1.
9. Enblock stain with UA	1% UA in 70% ethanol	30 minutes in the dark	
10.	80% ethanol	2 x 5 minutes	1. 2.
11.	95% ethanol	3 x 7 minutes	1. 2. 3.
12.	100% ethanol	3 x 7 minutes	1. 2. 3.
13. replace ethanol with propylene oxide	Propylene oxide	3 x 3 minutes	1. 2. 3.

Infiltration			
14. replace with propylene oxide/ epon mix (2:1)	2:1 propylene oxide: epon	2 hours, uncoverd	1.
15. replace with fresh 1:1 mixture *Transfer from 1.5ml tubes to BEEM capsules using forceps and needle*	1:1 propylene oxide: epon	1 hour	1.
16. replace with fresh 1:1 mixtures	1:1 propylene oxide: epon	1 hour	1.
17. change to fresh pure resin	100% epon	overnight	1.
Embedding			
18. replace with fresh Epon. Remove air bubbles if enter BEEM with the resin, add computer- printed labels	100% epon		1.
19. cure in 40C oven		40C oven for 24 hours	1.
20. transfer to 60C oven		60C oven for 48 hours	1.
21. remove from beam capsules, invert block (cells-side up) and let sit in 60C oven for additional 8 hrs before sectioning		60C for 8 hours	1.
*If the cell pellet is large, you can embed it by breaking it into small pieces after the final stage of infiltration. If treated carefully, the pellet can be flat-embedded, allowing sections to be made through which ever part of the pellet is required.			