PROTOCOL:

Biological Processing Steps for TEM

KIRSCH NOTES
PROTOCOLS & TIPS FOR MICROSCOPY

Chemical processing of biological tissues for microscopic examination has evolved to keep pace with increased levels of detail seen with newer technologies. From simple formalin fixation, paraffin embedment and 0.5 mm sections for OLM into a multichemical, epoxy resin embedment and 60 nm sections for TEM. Even this well-established TEM process has evolved further with the use of microwaves to significantly decrease the time and potential processing artifacts. This generalized procedure gives the acceptable changes, concentrations and times for both bench top and microwave processing.

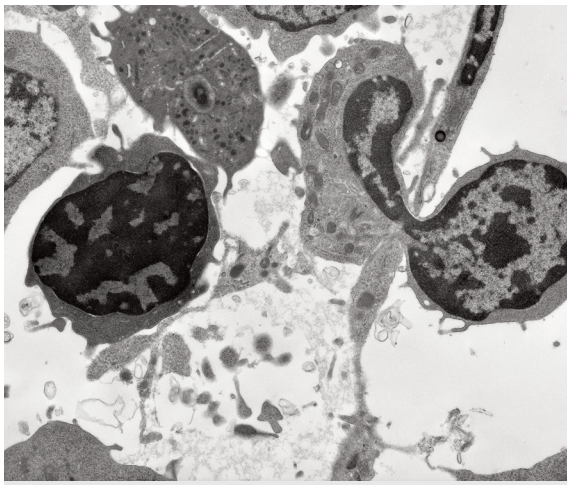
Setup requirements

- Program microwave for desired processing times.
- Get ORGANIZED!! Have equipment and solutions ready.
- Samples should be placed on a rotator when using Benchtop processing.
- Setup vacuum chamber and/or agitation, if needed.

All of the following steps can be carried out in the 1.7 ml microfuge tubes, scintillation vials, or Petri dishes.

NOTE: Acetone or propylene oxide (PO) cannot be used for dehydration if plastic Petri dishes are used.

Step			Temp.	Microwave	Lynx II/Benchtop
1.	Initial fixation (Karnovsky's)		37° C	2:30 min	2 hr.
2.	Buffer rinse 3 changes		37° C	60 sec. ea.	10 min. ea.
3.	2-4% OsO4 in DI water		37° C	2:30 min	2 hr.
	(Sometimes 2% Potassium Permanganate in DI is used for plants and bacteria.)				
4.	Water rinse **3 changes**		**37° C**	**60 sec. ea.**	**10 min. ea.**
NOTE: If using LR White, Acetone or PO can not be used, only ETOH!					
5.	Dehydration	50%	45° C	60 sec.	10 min.
	(Using either:	70%	45° C	60 sec.	10 min.
	ETOH, Acetone	80%	45° C	60 sec.	10 min.
	or Acetonitrile)	90%	45° C	60 sec.	10 min.
	2 changes	**100%**	**45° C**	**60 sec. ea**	**10 min. ea**
NOTE: Separate the SEM for CPD or HMDS from the TEM samples at this stage if necessary.					
(ETOH : Acetone OR		1:1	45° C	60 sec.	5 min.
ETOH : Acetonitrile OR		100%	45° C	60 sec.	5 min.
ETOH : PO)					
6.	Infiltration — ETOH, Acetone, or PO:Resin				
	Plant	3:1	50° C	15 min.	30 min.
		2:1	50° C	5 min.	1 hr.
		1:1	50° C	15 min.	1 hr.
	100% resin.	**2 changes**	**50° C**	**15 min. ea.**	**1 hr. ea**
7.	Embed in capsules and polymerize over night at 70° C				
8.	Or store in freezer in 100% resin until time for Embedment.				



Bone Marrow: Transmission electron microscope image of a thin section cut through an area of bone marrow area near the cartilage/bone interface in a mouse kneecap. Image shows small opening in the thin endothelium of the vascular sinus wall, where a blood cell is crossing the thin vascular sinus wall and into the sinus lumen.

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EMS Catalog supplies used	Cat. No.
Buffers	
Phosphate	**19340-72**
Cacodylate	**11652**
Fixatives	
Karnovsky's Fixative EM Grade	**15732-10**
2% OsO_4 Aqueous Solution	**19172**
Dehydrants	
Ethanol	**15055**
Acetone	**10015**
Propylene Oxide	**20401**
Acetonitrile	**10020**
Resins	
Embed 812	**14120**
Spurr's	**14300**
LR White	**14383**
LYNX II Automated Tissue Processor	**L12600**

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