EMS Microscopy Academy

Endless Possibilities ...

Kirsch notes — Biological Processing Steps for TEM

Biological Processing Steps for TEM

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 μm 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.

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% OsO_4 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.

EMS Catalog Supplies listed on back

EMS Catalog Supplies Used

Buffers

Phosphate	19340-72
Cacodylate	11652

Fixatives

Karnovsky's Fixative EM Grade	15732-10
2% OsO_4 Aqueous Solution	19172

Dehydrants

Acetone	10015
Ethanol	15055
Propylene Oxide	20401
Acetonitrile	10020

Resins

Embed 812	14120
Spurr's	14300
LR White	14383

Lynx II - Automated Tissue Processor - Catalog #L12600

Electron Microscopy Sciences | 1560 Industry Road | Hatfield, PA 19440
P 215-412-8400 | F 215-412-8450 | info@emsdiasum.com