



Endless Possibilities ...

SEM Biological HMDS Processing

Processing biological samples for secondary electron (SE) sampling in the Scanning Electron Microscope (SEM) requires the removal of fluids from their matrix. This is typically done using Critical Point Drying (CPD) or Freeze Drying (FD). Hexamethyldisilazane (HMDS), a chemical with extremely low surface tension, is a great alternative approach to CPD or FD for SEM samples. HMDS's low surface tension allows previously fixed and dehydrated samples infiltrated with HMDS to air dry without the artifacts typically associated with air drying.

Set Up

- **Get ORGANIZED!!** Have equipment and solutions ready to go because this process moves rapidly!
- Setup requirements for microwave if appropriate.
- Program microwave for desired processing times.
- Setup water load and reference temp probe.
- Setup vacuum chamber and/or agitation if needed.

EMS Catalog Supplies

HMDS 16700

The Procedure

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

STEP	Temperature	Time for Method Used	
		Microwave	Bench Top (ambient)
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.
5. Ethanol dehydration a. 50%	45°C	60 sec.	10 min.
b. 70%	45°C	60 sec.	10 min.
c. 80%	45°C	60 sec.	10 min.
d. 90%	45°C	60 sec.	10 min.
e. 100%	45°C	60 sec.	10 min.
f. 100%	45°C	60 sec.	10 min.
Transfer to Critical Point Drying holders immersed in ETOH if appropriate			
6. Infiltration – ETOH : HMDS		No Microwave HMDS	
Plant 3:1	Room Temperature		15 min.
2:1	Room Temperature		30 min.
1:1	Room Temperature		30 min.
100% HMDS	Room Temperature		30 min.
100% HMDS	Allow to evaporate in fume hood with no fan on overnight		