

Biological sample fixation protocol for SEM

This protocol will take 5-7 hours.

The EM Center maintains stocks of all necessary reagents.

1. Fix the tissue pieces in 0.1M cacodylate buffered (pH 7.2) 2.5% (or 3% for thicker or denser specimens) glutaraldehyde for 3-4 hrs at room temperature. This step can also be performed overnight.
 2. Wash several times in 0.1M cacodylate buffer (pH 7.2).
 3. Post-fix in 0.1M cacodylate buffered (pH 7.2) 1% (or 2%) osmium tetroxide for 1-1.5 hrs at 4°C.
 4. Wash 3 times in 0.1M cacodylate buffer (pH 7.2).
 5. Dehydrate in a series of ethanol (50%, 70%, 80%, 95%) for 10 minutes each.
 6. Dehydrate twice in 100% ethanol (10 minutes each).
- NOTE: DO NOT USE ACETONE!
7. Transfer your samples into microporous vials and immerse the vials in ethanol.
 8. Place the vials in the critical point dryer and critically dry before gold coating. You can also air dry instead of critical point drying, however critical point drying is recommended
 9. Mount your samples on a SEM stub and gold coat.
 10. Samples are now ready for SEM.