

Suggested biological sample fixation protocol for TEM

This protocol takes approximately 5 days.

The EM Center maintains stocks of all necessary reagents.

1. Fix the tissue pieces in 0.1M cacodylate buffered (pH 7.4) 2.5% (or 3% for thick or dense specimens) glutaraldehyde overnight at room temperature.
2. Wash several times in 0.1M cacodylate buffer (pH7.4).
3. Post-fix in 0.1M cacodylate buffered (pH 7.4) 1% (or 2%) osmium tetroxide for 1-1.5 hrs at 4°C.
→NOTE: Osmium tetroxide is extremely toxic. Avoid any contact with the skin. OsO₄ must be disposed of properly. Please dispose of OsO₄ in properly labeled containers.
4. Wash 3 times in 0.1 M cacodylate buffer (pH 7.4).
→Note: The EMBED 812 is in the -20°C freezer. Bring it up to room temp before use (step 8).
5. Dehydrate in a series of ethanol (50%, 70%, 80%, 95%) for 10 minutes each.
6. Dehydrate twice in 100% ethanol (10 minutes each).
7. Incubate in acetone twice for 5 minutes each.
8. Add EMBED 812 (medium mixture) and acetone 1:1 and incubate overnight on a rotator at room temp.
9. On the following morning, add EMBED 812 and acetone 3:1 and incubate for 5 hrs on a rotator at room temperature.
10. Add 100% EMBED 812 and incubate overnight on a rotator at room temperature.
11. On the following day, embed in fresh 100% EMBED 812 by placing the specimen into an embedding block mold and adding the fresh EMBED 812. Successful trimming of the cured EMBED 812 block requires an appropriate placement of the specimen within the block. Move the specimen so that it is near the top of the narrow end of the block using a small wooden stick. Place the blocks into an oven and let the blocks cure for 24 hrs at 60°C.

Table 1. Reagents typically prepared in advance by EM staff

Reagent	Location
50% glutaraldehyde	4°C fridge
0.2 M cacodylate buffer	4°C fridge
2 % OsO ₄	Please see staff
EMBED medium mixture	-20°C fridge