

Preparation of Rodent Temporal Bones for Scanning Electron Microscopy

1. Fix tissue in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer pH 7.3 with 3mM CaCl₂ (2 - 3 hours minimum, but better if done for 24-48 hrs. can be done at room temp or 4C, ideally in a sealed container in a fume hood). If possible, perfusion through the round window and/or an open fenestra at the helicotrema)
2. Rinse in 0.1M cacodylate buffer (3 x 5 min, room temp)
3. Fix in 1% OsO₄ in 0.1M cacodylate. (30 – 60 min, room temp, in fume hood)
4. Rinse in cacodylate buffer (3 x 2 min), then transfer to ddH₂O (2 x 5 min, room temp)
5. Remove most of the H₂O, add saturated aqueous solution of thiocarbohydrazide (0.5% in water, stirred, filtered just before use) dropwise, if solution turns opaque brown, re-rinse in ddH₂O, if solution is translucent or clear, keep adding until tissue is immersed (20-30 min., room temp)
6. Rinse in ddH₂O (3 x 5 min)
7. Fix in 1% OsO₄ in 0.1M cacodylate. (30 min, room temp)
8. Rinse in ddH₂O (3 x 5 min)
9. Repeat steps 5-7 (if you have the time, you should repeat these steps again for a total of 3 iterations of OTO).
10. Dehydrate in ethanol series to anhydrous 100% ethanol
 - 25% EtOH, 5 min
 - 50% EtOH, 5-10 min
 - 75% EtOH, 5-10 min
 - 95% EtOH, 5-10 min
 - 100% EtOH, 3 X 10 min
11. At this point, dissect away the bone to reveal the sensory tissue. Then Critical point dry using liquid CO₂. Proceed immediately to mounting and sputter coating.
- 11.5 (OPTIONAL) If critical point drying is unavailable, samples can be solvent dried in hexamethyldisilazane (HMDS). Add enough HMDS to completely cover your sample and let sit in a closed well plate overnight in the fume hood. Evaporate residual HMDS from the sample in a 37C oven for 1-3 hours. Proceed immediately to mounting and sputter coating.
12. Mount on SEM stubs using double sided adhesive carbon discs.
13. Sputter coat (between 5 – 15 nm, depending on type of coating being used and structures to be visualized).

Supplies:

Glutaraldehyde solution (25%): 18426 from Ted Pella

Sodium cacodylate

CaCl₂

OsO₄; 4% aqueous solution – 18459 (10x2ml) from Ted Pella

Thiocarbohydrazide (from Sigma; No. T-2134)

Anhydrous ethanol

Specimen support stubs for SEM (12.5 mm: 16232, pack of 50, from Ted Pella

PELCO Carbon conductive tabs, double sided (16084-1 from Ted Pella)