

### TEM Protocol for Cells Suspended in Media

1. Pellet the cells, pipette off media, then incubate the cells with phosphate buffered saline (PBS) containing 2% paraformaldehyde and 2.5% glutaraldehyde.
2. Incubate the cells with fixative for 1h at room temperature (RT)
3. Wash the fixed cells with PBS (2×, 5 min).
4. Pellet the cells (200g × 5 min) then incubate them with 1% osmium tetroxide, 1% potassium ferricyanide, and 0.2% tannic acid in PBS. in phosphate buffer.
5. Rinse the cells with DI water (2×, 5 min).
6. Incubate the cells with 25% ethanol (1×, 10 min), 50% ethanol containing 1% uranyl acetate (for *en bloc* staining; 1×, 60 min), 70% ethanol (1×, 10 min), 95% ethanol (3×, 10 min), then 100% ethanol (3×, 10 min).
7. Incubate the pellet with 100% propylene oxide (3×, 10 min each) then 1:1 propylene oxide: epoxy resin (overnight in the hood on a rotatory mixer).
8. Incubate cell pellets with a 1:2 mixture of propylene oxide: epoxy resin on a rotary mixer for 6h at room temperature.
9. Incubate with 100% epoxy resin overnight in the hood on a rotator.
10. Incubate with fresh 100% epoxy resin for 2h in the hood on a rotator.
11. Polymerize in an oven at 70°C for 36h.
12. Remove the sample from the centrifuge tube, cut off the pellet, and place it in the tip of a BEEM capsule.
13. Fill the BEEM capsule with 100% epoxy resin and polymerize in an oven at 70°C for at least 12h.
14. Section resin blocks containing cells of interest with an ultramicrotome.
15. Collect sections on formvar- and carbon-coated 200 mesh copper EM grids.
16. Stain sections with 1% filtered uranyl acetate (5 min) then Reynold's lead citrate (2 min).
17. Image the grids.

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|-------------|---------|
| Epoxy Resin |         |
| Embed 812   | 10.0 g  |
| DDSA        | 4.5 g   |
| NMA         | 6.0 g   |
| DMP-30      | 0.35 ml |

Modified from Graham, L., & Orenstein, J.M. (2007). Processing tissue and cells for transmission electron microscopy in diagnostic pathology and research. *Nature Protocols*, 2(10), 2439-2450.