

TEM Protocol for Cells Attached to a Substrate

1. Pour off medium and replace with phosphate buffer containing 2% paraformaldehyde + 2.5% glutaraldehyde.
2. Incubate for 1h at room temperature (RT).
3. After 1h fixation, wash the fixed cells twice with phosphate buffer. Degenerating and necrotic cells will generally spontaneously lift off the substrate and can be discarded if so desired.
4. Incubate with phosphate buffer containing 1% osmium tetroxide, 1.5% potassium ferricyanide, and 0.2% tannic acid for 1h at RT in the dark.
5. Dehydrate in an ascending series of alcohols: 25% ethanol (1×, 10 min), 50% ethanol containing 1% uranyl acetate (for *en bloc* staining; 1×, 60 min), 75% ethanol (1×, 10 min), 95% ethanol (3×, 10 min), 100% ethanol (3×, 10 min).
6. Remove the final 100% ethanol from the wells using a glass pipette, replace it with propylene oxide, and leave it for 30s.
7. Draw the propylene oxide up and down in the pipette and transfer the cells to a microfuge tube. The cells will come off in sheets of transparent film. Centrifuge at 9,500g at room temperature for 5 min.
8. Subject pellets to two more changes of propylene oxide (10 min each), centrifuging each time.
9. Incubate cell pellets with a 1:1 mixture of propylene oxide: epoxy resin on a rotary mixer for 6h at room temperature.
10. Incubate cell pellets with a 1:2 mixture of propylene oxide: epoxy resin on a rotary mixer for 6h at room temperature.
11. Incubate cell pellets with 100% epoxy resin on a rotary mixer for 6h at room temperature.
12. Incubate cell pellets with 100% epoxy resin on a rotary mixer for 2h at room temperature.
13. Polymerize in an oven at 70°C for at least 12h.
14. Remove the sample from the centrifuge tube, cut off the pellet, and place it in the tip of a BEEM capsule.
15. Fill the BEEM capsule with 100% epoxy resin and polymerize in an oven at 70°C for at least 12h.

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.