PROCESSING CELLS GROWN IN CULTURE FOR ELECTRON MICROSCOPY

Supplies:

1. Buffered aldehyde fixative, either 4% paraformaldehyde in 0.1 M phosphate buffer, or 2.5% glutaraldeyde in 0.1M buffer,or a mixture of the two fixatives (i.e. Karnovsky's)
2. Buffered 1% osmium tetroxide
3. Additional 0.1M buffer. Most commonly used: Phosphate and Cacodylate
   The Core recommends using 0.1M sodium phosphate buffer, pH 7.2
4. A graded series of acetone or ethanol: 10%, 35%, 50%, 70%, 95% and 100%
5. Prepared embedding mix, or the components for the mix (see embedding mix recipes)
6. Premade blank BEEM capsules to glue samples onto, and SuperGlue
7. Glass 24mL snap-cap specimen vials and plastic 1.5mL centrifuge tubes
8. A supply of glass and plastic pipettes
9. A supply of single edged razor blades
10. A rubber policeman
11. Plastic waste containers for waste fixatives, waste solvent and waste plastic
12. Two ovens, set to 50°C and 70°C, and a vacuum container or Bell jar

1. If cells are in a flask or Petri dish, they may be fixed *in situ*. The medium is removed from the cells and replaced with enough fixative to cover the cells to a depth of several millimeters. Or, if the cells are in suspension, loosely pellet them in a 1.5mL plastic microcentrifuge tube. Remove the supernatant (media) and replace it with enough fixative 4% paraformaldehyde in 0.1 M phosphate buffer (best for a sample that is to undergo processing for immunochemistry or immuno fluorescence prior to TEM processing) to fill the tube. The fixative should be warmed up to 37°C (or at least room temperature) before adding it to the cells to prevent cell contraction. Cap the tube and invert it to resuspend the cells. **Post-fixing the cells overnight at 4°C with 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.2. is customarily done on the portion of a sample that is intended only for EM processing but not necessary in most cases.** Care must be taken to put Petri dishes or flasks in a closed container before refrigerating the samples to prevent fixative from contaminating the refrigerator. Use a fume hood and wear gloves when processing samples for electron microscopy! On the following day, the cells fixed *in situ* should be rinsed in 0.1M sodium phosphate buffer several times, and then be scraped off their dish with a rubber policeman and pelleted loosely in 1.5 mL microcentrifuge tubes. Ideally, 6 million cells should be harvested per sample for electron microscopy to account for cell loss during processing. This step can be done in your lab. (We can furnish fixative at supplies charge as a convenience.)

   The steps following, can be done at Core Lab (required for training but optional for regular users.) For subsequent centrifugations, cells are to be pelleted at 1000 RPM for 3 minutes in all steps up to 100% acetone. Ideally, processing the samples should begin at 8 A.M. on the second day.

2. The cells are rinsed 3 times in 0.1M sodium phosphate buffer for 5 minutes each, with centrifugation between rinses. The cells are post-fixed with 1% osmium tetroxide in the above buffer at 4°C for 1 hour.

Again, the capped samples must be put in a closed container to prevent fixative contamination of the refrigerator. All used buffer and fixative waste is to be put into properly labeled waste containers.

3. The cells are rinsed 3 times in the above buffer, 5 minutes each, at 4°C. Recap the tubes between rinses.

4. The cells are dehydrated in a graded series of ethanol or acetone, 10%, 35%, 50%, 70%, and 95% (in water), for 5 minutes each at room temperature. Recap the tubes between steps.
   All waste is to be put into a properly labeled waste container. Keep the acetone changes closed until needed. Acetone will absorb water from the air, and samples that are not completely dehydrated will not polymerize properly.

5. The cells are resuspended in 100% acetone. This step is repeated 3 times, 10 minutes each, with pelleting at 2000 RPM between each step. Recap the tubes between steps.

6. Following centrifugation, the cells are resuspended in a 50:50 mix of acetone:embedding medium (LX112 resin mix, see embedding medium recipe), uncapped, under vacuum for 1 hour. All waste embedding medium is to be placed into a properly labeled waste container. This waste is to be polymerized at 50°C for several days before discarding the plastic.

7. Pellet the cells at 3000 RPM, and resuspend them in 100% embedding mix. Infiltrate the cells in embedding mix for 1 hour under vacuum. Repeat this step 2 more times, pelleting the cells at 7800 RPM for 5 minutes between each step. Following each change of embedding medium, resuspended the cells by stirring them in the fresh embedding mix with a plastic stirrer. Protect the embedding mix from absorbing water from the room air. Keep the mix capped until needed. Embedding mix that absorb water will not polymerize properly.

8. Infiltrate the cells in a last change of embedding medium for 3-4 hours under vacuum, then pellet them at 7800 RPM for 10-15 minutes. Place the samples in microfuge tubes, uncapped, in a 70°C oven overnight to polymerize the embedding mix.

9. Trimming block and thick sectioning for 1% toluidine blue stain on glass slides followed by thin sections on copper grids (3 grids)

10. Staining grids with 8% aqueous uranyl acetate and with Reynolds lead citrate.

11. Thin sections can now be observed and photographed with the electron microscope.