Basic TEM Processing Procedure

Here is a sample procedure that will work for most tissue preparations. Please see the facility manager for detailed help with your samples specifically.

Materials
- Karnovsky’s fixative (4% formaldehyde 2.5% glutaraldehyde)
- 0.1M Phosphate Buffer
  - make a 0.2M stock of both monobasic and dibasic sodium phosphate. Using a pH meter, adjust the pH, then dilute to 0.1M for a working solution. This should be made fresh!
- 2-4% stock of aqueous osmium tetroxide
- 50, 70, 95 and 100% ethanol
- propylene oxide
- Spurr’s resin

Procedure – can be done in either a glass scintillation jar but if the sample is small enough, it works quite nicely in an eppendorf tube. The tissues should be kept moving through steps 1-5 on a shaker.

1. Immerse samples that are no more than 2 mm (necessary for osmium infiltration) thick in Karnovsky’s fixative as quickly as possible. The fixative should be 10 times greater in volume than that of the sample. Fix 1 – 2 hours at room temperature or overnight.
2. Rinse tissue three times 15 minutes each in 0.1 M phosphate buffer.
3. Secondary fixation is done in 1% OsO₄/0.1 M phosphate buffer for 2 hr at room temperature.
4. Rinse tissue in distilled water or 0.1M phosphate buffer three times 15 min each.
5. Dehydrate sample by passing through an ascending alcohol series. The ethanol volume should be 5 – 10 times that of the sample volume.
   a. 50% EtOH 15 min.
   b. 70% EtOH 15 min. (samples can be left overnight)
   c. 95% EtOH 15 min.
   d. 100% EtOH 15 min.
   e. 100% EtOH 15 min.
   f. 100% propylene oxide 10 min.
   g. 100% propylene oxide 10 min.
6. Infiltrate with Spurr’s resin
   a. 1:1 resin:propylene oxide 1 hour
   b. 100% resin overnight
   c. Fresh 100% resin; put into molds with label
7. Polymerize in 70°C oven overnight