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Tips for Assuring Good Electron Microscopy Results

1. Primary Fixation of Samples:

a. We recommend McDowell's and Trump's 4F:1G* as a primary fixative, though 2-3% buffered glutaraldehyde at physiological pH should work quite well.
b. The sample should be no more than **1 mm thick** in at least one dimension, since most primary and post-fixatives do not penetrate much more than 0.5 mm one hour, and autolysis can occur if primary fixation takes longer than 1 hr.
c. The fixative fluid (and any other processing fluids) should be about **5-10X the volume of the sample.**
d. Do **not** take EM samples from large pieces of previously fixed tissues any farther than 1 mm from the surface (fixative will not have penetrated adequately).
e. **Never let your samples become dry on the surface.**
f. If fixing plant leaves, it is useful to subject them to vacuum (approximately 15 lbs./in$^{2)}$ for several minutes to help remove air trapped by trichomes so that the leaves are more thoroughly wetted (and fixed) by the fixative solution.

2. Buffers: For most samples, a 0.1 M phosphate buffer (or cacodylate buffer for cytochemical work employing charged molecules, or when working with marine samples) at physiological pH for your tissue/cells is appropriate*.

3. Dehydration: Standard ethanolic dehydration series followed by passage through 100% acetone* will work well for most samples going into epoxide resins. **Propylene oxide is neither needed nor recommended, due to its toxicity.** For **acrylic resins**, omit the acetone steps to avoid poor polymerization.

4. Processing Particulate Samples: If your sample consists of suspended cells (yeasts, bacteria, protozoans, etc.) or small metazoans (e.g., shrimp embryos), agar-embed them* after washing out the primary fixative with buffer.

5. Sample Storage: If you must store samples after primary fixation, but before processing, store them in the primary fixative in a refrigerator. This will decrease the chance of fungal or bacterial growth destroying the sample (which can happen to tissues stored in buffer) and will decrease the activity of hydrolytic enzymes that have leaked from lysosomes after primary fixation and which can contribute to leaching of cellular consituents during processing.

6. Embedding Molds: Use either BEEM-type capsules or silicone flat-embedding molds for epoxide embedment and gelatine capsules for acrylic resins. **The use of non-standard embedding molds such as microfuge tubes makes it extremely hard**

to hold them in the ultramicrotome chucks, leading to chatter and poor sections.

7. **Choice of Embedding Media:** We recommend Spurr resin* for standard epoxide embedment for structural studies. It has the lowest viscosity of the widely-available resins, which allows it to penetrate samples most easily. If immunological work is anticipated, acrylic resins are a better choice (see point 6).

8. **Labeling blocks:** If you are labeling your own blocks, laser-jet createdlabels will work for **epoxide** resins, but they should be pre-dried in the polymerizing oven before use to avoid water contamination of the resin. For **acrylic** resins, pencil-written labels are preferred, since laser-jet labels tend to run and smear in the resin. **Do not label molds externally:** All labels should be placed within the resins in the molds, so that the information cannot be separated from the sample. ***Ink-jet labels tend to run in any resin and should not be used.***

9. **Special Procedures:** If immunological or cytochemical work is needed, or any methods other than those outlined in **Dykstra and Reuss, 2003*** need to be employed, please contact the LAELOM in advance so that we can be sure to be able to produce the quality of product necessary to both you and us.

Dykstra, M.J., and Reuss, L.E. 2003. *Biological Electron Microscopy: Theory, Techniques and Troubleshooting.* 2nd Edition. Kluwer Academic/Plenum Publishers, N.Y. 534 p.