

Laboratory for Advanced Electron and Light Optical Methods

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A Routine Fixation and Embedding Schedule for Transmission Electron Microscopy Samples (Tissues or Cell Suspensions)

(from Dykstra, M.J., and L.E. Reuss. 2003. *Biological Electron Microscopy: Theory, Techniques and Troubleshooting*. Kluwer Academic Press, N.Y. 534 p.)

1. Applications and Objectives: This technique can be applied to **viruses, bacteria, protozoans, plants, fungi, and animal tissues or cells**. Specific procedural instructions for handling microorganisms or cells in culture (monolayers *in situ* or suspended) are provided as separate protocols later in this manual. **Samples can be stored in the primary fixative for several years in a refrigerator**. The primary fixative is particularly useful for collecting samples in the field for further processing later in an electron microscopy laboratory. Trump's 4F:1G is equally useful for perfusion as well as immersion fixation of samples. **Samples fixed in 4F:1G can be embedded in paraffin and stained with all conventional histological techniques and the blocks do not have the brittleness associated with tissues fixed in 2% or higher glutaraldehyde concentrations.**

2. Materials Needed:

4F:1G fixative **
0.2 M and 0.1 M Sorenson's sodium phosphate buffer, pH 7.2-7.4
2% aqueous osmium
100% ethanol (to make the dilution series)
100% acetone (transitional solvent)
Spurr resin (6.3 g DER recipe)

3. Procedure:

- 3.1. Immerse samples that are no more than 1 mm thick in at least one dimension in 4F:1G fixative as quickly as possible. The fixative volume should be 5-10 X that of the sample volume. Fix for 1-2 hrs at room temperature (see cautionary statement below about storage of fixed tissues).
- 3.2. Rinse tissue two times (15 min each) in 0.1 M Sorenson's sodium phosphate buffer at pH 7.2-7.4.
- 3.3. Post-fix sample in 1% osmium tetroxide/0.1 M phosphate buffer for 1 hr at room temperature (made by mixing equal volumes of 2% aqueous osmium and 0.2 M Sorenson's sodium phosphate buffer, pH 7.2-7.4).
- 3.4. Rinse tissue in distilled water two times (5 min each).

3.5. Dehydrate sample by passing it through the following alcohol series (always using fluid volumes at least 10X greater than the sample volume):

50% ethanol 15 min
75% ethanol 15 min (**If necessary, samples can be left overnight at 4° C**)
95% ethanol 15 min, two times
100% ethanol 30 min, two times
100% acetone 10 min, two times

3.6. Infiltrate with Spurr resin (6.3 recipe)

Spurr:100% acetone (1:1) 30 min
100% Spurr resin 60 min
100% Spurr resin 60 min
NEW 100% Spurr resin; put in appropriate molds with labels

3.7. Polymerize in 70° C oven overnight to 3 days.

4. Results Expected: Tissues/cells will have nuclear envelopes with parallel membranes, mitochondria and endoplasmic reticulum with no signs of swelling, and cytoplasm and nucleoplasm with no signs of excessive protein extraction.

5. Cautionary Statements: The fixatives used should be handled under a fume hood because of their toxicity and should not come into contact with skin. Spurr resin is regarded as a carcinogen, and should be formulated, handled, and polymerized under a fume hood and should not come into contact with skin.

The primary fixative (4F:1G) is stable at 4° C for several months prior to use. Samples may be stored at the same temperature for several years without excessive extraction of cytoplasmic components.

All osmium and water washes following osmium must be collected in a container designated for osmium waste.

All plastic resins and diluted plastic resins must be collected in disposable plastic beakers for polymerization before disposal. **Do not put any resins down laboratory sinks.** The resins are considered to be carcinogenic, so **do not breathe the fumes or allow skin contact** with the resin or its components.

Used Spurr resin should be removed from sample vials with 5 1/4" Pasteur pipets and transferred to a disposable beaker kept under the fume hood. After several weeks, any remnants of acetone will have evaporated and the resin will have polymerized. At that time, the beaker containing solid resin can be disposed of in the trash. To add fresh resin to vials, plastic disposable pipets are used to make sure that pipets for removing waste resin are not confused with pipets containing fresh resin, leading to contamination of fresh resin.

Spurr resin is readily contaminated by water. Be careful to keep all containers of the fluid resin closed and free of water vapor. In some cases, breathing into vials containing resin or sneezing near resin in open containers has resulted in poorly polymerized, rubber-like blocks.

Labels are customarily prepared and placed in molds during the last 100% infiltration step and then placed in a 70° C oven until needed to assure that the labels and molds are moisture-free. See the instructions for Spurr resin formulation concerning filling molds to proper levels. If using BEEM capsules, remove the lids. If any of the epoxide resins contain residual amounts of the sample dehydration agents (ethanol, acetone), closing the caps of the BEEM capsules can result in retention of these agents, thereby preventing proper resin polymerization.

References:

McDowell, E.M., and B.F. Trump. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. 100:405.

Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31.

**** Formulation of 4F:1G fixative:**

This fixative is a mixture containing 4% formaldehyde and 1% glutaraldehyde in a monobasic phosphate buffer with a final pH of 7.2-7.4 and a final osmolality of 176 mosmol. To make 100 ml of 4F:1G, mix the following in the order listed:

86 ml distilled water
10 ml Fisher F-79 (37-40% formaldehyde)*
4 ml 25% biological grade glutaraldehyde
1.16 g NaH₂PO₄H₂O
0.27 g NaOH
(Stir while adding components)

*Substituting formaldehyde made freshly from paraformaldehyde powder is recommended for cytochemical procedures to avoid any potential problems from the stabilizers added to typical 37-40% formaldehyde stocks. **As with all procedures calling for formaldehyde made from paraformaldehyde, it is recommended that the fixative be made just before use to lessen the chance that the formaldehyde re-polymerizes into paraformaldehyde.**

This fixative is strongly recommended for perfusions or immersion fixation of plants, animals, fungi, bacteria, viruses, and many protozoans. The fixative is stable at 4° C for at least 3 months before use and samples may be stored in the fixative at the same temperature for up to 18 years without serious damage (see Dykstra and Reuss, 2003). In addition, the glutaraldehyde concentration is low enough that tissues embedded in paraffin still section and stain well (glutaraldehyde concentrations of 2% and above result in brittle paraffin blocks and non-specific periodic acid-Schiff's reagent staining), so this is an ideal fixative if a sample is used for both light and electron microscopy.