

Laboratory for Advanced Electron and Light Optical Methods

College of Veterinary Medicine
4700 Hillsborough Street
North Carolina State University
Raleigh, NC 27606
Tel: 919-513-6202
Fax: 919-513-6464
Email: Michael_Dykstra@ncsu.edu
www.cvm.ncsu.edu/research/laelom

Fixative Preparation

I. McDowell's and Trump's 4F:1G

- A. **Purpose:** This fixative is the first choice in the LAELOM because it gives good ultrastructural preservation, produces samples that can be processed for histological preparation without producing brittle paraffin-embedded samples and also remains normally reactive with standard histological stains, including PAS. In addition, tissues have been stored in this fixative in the LAELOM under refrigeration for over 12 years without notable degradation of tissue quality. **Thus, this fixative is suitable for both electron microscopy and light microscopy preparations.** For examples of tissues fixed and stored in 4F:1G and further explanations of the utility of this fixative, see: Dykstra, M.J. and L.E. Reuss. 2003. *Biological Electron Microscopy: Theory, Techniques and Troubleshooting*. 2nd Edition. Kluwer Academic Press, NY.
- B. **Procedure:** This fixative contains 4% formaldehyde and 1% glutaraldehyde in a phosphate buffer, with an osmolarity of 176 mOsM and a pH of 7.2-7.4.

Mix the following, in the order listed:

86 ml distilled water
10 ml Fisher F-79 37-40% formaldehyde (or suitable substitute)
4 ml 25% biological grade glutaraldehyde
1.16 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$
0.27 g NaOH

Stir while adding the components and check the pH when done. Use 0.1 N NaOH to adjust the pH, if necessary.

- C. **A Modified 4F:1G Recipe** can be employed for cytochemical procedures if the stabilizing compounds in 37-40% formaldehyde are contraindicated:

Procedure: To make a 4% formaldehyde/1% glutaraldehyde fixative in 0.1 M sodium phosphate buffer utilizing **paraformaldehyde powder**:

1. Bring 120 ml distilled water in a beaker to a temperature of about 68° C on a hot plate **under the fume hood**.
2. Add 10 g of paraformaldehyde powder while stirring.
3. Add 1 N NaOH dropwise until the solution clears.
4. Cover container and cool to room temperature (it may be placed in the refrigerator until cooled).
5. Add 10 ml of 25% glutaraldehyde
6. Bring the volume up to 250 ml with 0.2 M Sorenson's phosphate buffer (pH 7.2-7.4) or 0.2 M sodium cacodylate buffer at the same pH (the latter for procedures where reactive compounds with a positive charge are to be encountered).
7. The final pH should be approximately 7.3 (**check it**), and the osmolarity approximately 1583 mOsM.

II. Preparation of a Modified Karnovsky's Fixative

- A. **Purpose:** Karnovsky (M.J. Karnovsky. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* 27:137A) originally designed this fixative for perfusion of mammalian tissues and formulated it as a sodium cacodylate-buffered (80mM), calcium chloride-enhanced (5 mM) mixture of 5% glutaraldehyde and 4% formaldehyde (prepared from paraformaldehyde powder). The original recipe produced a hypertonic medium (2010 mOsM) that was so hypertonic that most investigators made it up at half-strength. Over the years, investigators have called fixatives "Karnovsky's" that were made up without the calcium chloride, with phosphate buffer rather than sodium cacodylate buffer, and various concentrations of formaldehyde and glutaraldehyde. **Unless the specific recipe is listed in a publication where the authors claim to use "Karnovsky's", do not assume they are using the original formulation. They rarely are doing so.**
- B. **Procedure:** Remember, this procedure is for a **Modified Karnovsky's**. The final solution contains 2% formaldehyde and 2.5% glutaraldehyde in a sodium phosphate buffer.
1. **Under the fume hood**, bring 25 ml of distilled water to approximately 68° C and add 1.0 g paraformaldehyde powder while stirring.
 2. Add 1-3 drops of 1 N NaOH and stir the solution until it clears.
 3. Cool the solution to room temperature.
 4. Add 5 ml of 25% glutaraldehyde.
 5. Add Sorenson's 0.2 M sodium phosphate buffer at pH 7.2-7.4 to bring the volume to 50 ml.

This fixative should not be kept for more than two weeks before use because the formaldehyde component will begin repolymerizing into paraformaldehyde. Store the solution at 4° C.

III. Preparation of 16% Formaldehyde from Paraformaldehyde

- A. **Purpose:** This procedure is useful if this fixative will be diluted with other fixative components, particularly for cytochemical procedures.

B. Procedure:

1. **Under the fume hood**, bring 50 ml of distilled water to approximately 68° C.
2. Add 8 g of paraformaldehyde powder while stirring.
3. Add 1 N NaOH dropwise, with stirring, until the solution clears.
4. Cool to room temperature.

IV. Preparation of Formaldehyde/Glutaraldehyde Mixtures Suitable for Fixing Samples for Ultracryomicrotomy and Subsequent Immunolabeling Utilizing the Tokuyasu Method
(K.T. Tokuyasu. 1984. Immuno-cryoultramicrotomy. In: *Immunolabeling for electron microscopy*, J.M. Polak and I.M. Varndell (eds.). Elsevier, NY. , Chapter 6).

Fixative	ml of 16% formaldehyde	ml of distilled water	ml of 0.4% glutaraldehyde	ml of 0.2 M phosphate buffer (see SOP 7.0)	Final Concentration (Form./Glut)
A	5	5	-	10	4%/0%
B	5	-	5	10	4%/0.1%
C	2.5	7.5	-	10	2%/0%
D	2.5	2.5	5	10	2%/0.1%