

Formaldehyde-Glutaraldehyde-Picric Acid (FGP) Fix

Warning: Picric Acid (trinitrophenol) is a potential explosive. Information on the safe handling of picric acid can be found [here](#) and [here](#).

Materials

Paraformaldehyde, Granular (Electron Microscopy Sciences (EMS), CAT #19208)

0.1N Sodium hydroxide (EMS, CAT #21170-01)

Picric acid (EMS, CAT #19550)

0.2M Sodium Cacodylate buffer (EMS, CAT #11650)

Calcium chloride, dihydrate (EMS, CAT #12340)

25% Glutaraldehyde, EM grade (EMS, CAT #16210)

50ml beaker

50ml centrifuge tube

Deionized water

Prepare FP Stock (“2/3 FP”) in hood

1. Heat the following ingredients in a 50ml beaker on a hot plate, until the mixture begins to steam (**do not boil**):

1.0g paraformaldehyde

7.58ml deionized water

2. Add 2-4 drops of 0.1N sodium hydroxide to dissolve & clear the solution

3. Add 1.92ml saturated picric acid solution* while the above solution is still hot.

4. After the picric acid has dissolved, add 22ml of 0.2M sodium cacodylate buffer and 0.5ml of 5% calcium chloride

5. Adjust the pH to between 7.3 and 7.4 with 0.1N sodium hydroxide as necessary.

6. Store the stock solution in the refrigerator (keeps for months).

FP Stock final concentration: 3.12% formaldehyde, 0.08% picric acid, 0.14M sodium cacodylate buffer, 0.08% calcium chloride.

For Tissue Fixation, use “2/3 FGP”

1. 4ml FP stock (from above)

2. Add 1ml 25% glutaraldehyde (add just before use because glutaraldehyde only lasts a few days after thawing).

3. Tissues are immersed in fixative for 1-2h at room temp, rinsed several times with the 0.2M cacodylate buffer (15 minutes to overnight, until solution runs clear), then post-fixed with osmium tetroxide (see [here](#)).

Final concentration: 2.50% formaldehyde, 5% glutaraldehyde, 0.06% picric acid, 0.1M sodium cacodylate buffer, 0.06% calcium chloride.

For Fixation of Thin Tissue or Tissue Culture Cells, use “1/3 FGP”

1. 4ml stock (from above)

2. Add 1ml 25% glutaraldehyde

3. Add 5 ml deionized water or 0.2M sodium cacodylate buffer
4. Tissues are immersed in fixative for 1-2h at room temp, rinsed several times with the 0.2M sodium cacodylate buffer (15 minutes to overnight, until solution runs clear), then post-fixed with osmium tetroxide (see [here](#)).

Final concentration: 1.25% formaldehyde, 2.5% glutaraldehyde, 0.03% picric acid, 0.05-0.16M sodium cacodylate buffer, 0.03% calcium chloride.

*Saturated picric acid is made by adding picric acid into deionized water until the yellow solution becomes saturated (when the picric acid solution is saturated there should be a visible precipitate). Saturated picric acid = 1.3%.

Picric acid preserves cytoplasmic ground substance (see Ito, S. & Karnovsky, M. J. (1968) Formaldehyde-glutaraldehyde fixatives containing trinitro compounds. *J Cell Biol* **39**:168A–169A)