

STANDARD FIXATIVES

0.172M Phosphate Buffer Stock (For 1 litre):

1. To 1000ml double distilled H₂O, add 6.82g NaH₂PO₄. Stir until it dissolves (goes in quickly).
2. Add 17.32g of Na₂HPO₄. Stir until dissolved (takes a bit longer).
3. Adjust pH to 7.2

To make 0.137M phosphate wash buffer (iso-osmotic):

1. Dilute 4 parts of 0.172M stock solution with 1 part distilled H₂O.

1% Paraformaldehyde, 1% Glutaraldehyde in 0.086M phosphate buffer

1. To \approx 450ml distilled H₂O, add 10.0g paraformaldehyde.
2. Heat solution on a hot plate to almost 80°C.
3. Clear with 5-10 drops of 1N NaOH. Let cool.
4. Add 500ml of 0.172M phosphate buffer stock, pH 7.2.
5. Add 14.3ml of 70% glutaraldehyde.
6. Bring final volume up to 1000ml.
7. Store in fridge at 4°C.

4% Osmium Tetroxide Stock Solution:

1. Using a hood and wearing gloves, break OsO₄ ampoules in a thick sided bottle. DANGEROUS OXIDIZER: avoid any contact!!

2. Add double distilled H₂O, seal the lid of the bottle with parafilm and leave in the hood at room temperature for a day or two until crystals dissolve. Invert or swirl the bottle every few hours. Final solution will be a pale yellow.

3. Store in fridge at 4°C. LABEL BOTTLE: CAUTION!!!

4. Over time the solution will start to turn from pale yellow to gray and ultimately to black. Only use when pale yellow.

5. Discard in properly labeled Toxic waste container.

For 25ml, dissolve 1.0g OsO₄ in 25ml dd H₂O.

2% OsO₄ in 0.086M phosphate buffer

Combine equal volumes of 4% OsO₄ stock with 0.172M phosphate buffer stock.

0.2M Sodium cacodylate stock buffer

For 500ml, add 21.40g of sodium cacodylate (CONTAINS As!!) to 500ml dd H₂O

4% paraformaldehyde in 0.1M sodium cacodylate buffer

For 1000ml:

1. Add 40.0g paraformaldehyde to 500ml of 0.2M sodium cacodylate stock buffer.

2. On a hot plate in the hood, heat mixture until paraformaldehyde dissolves and solution clears. (No NaOH is needed.)

3. Add about 400ml of dd H₂O.

4. Let solution cool.

5. Adjust pH to 7.4 using 1N HCl.
6. Pour solution into a graduated cylinder and bring volume up to 1 liter.
7. Store in fridge at 4°C.