4% Paraformaldehyde Fixative

1. Add deionized water to a flask and heat to 60° C, keeping below 70° C;
2. Add the paraformaldehyde, with constant stirring, continue stirring for 10 min;
3. Slowly add a few drops of 10 N NaOH to the cloudy mixture, stirring until the solution begins to clear;
4. Stir a few minutes longer, adding more NaOH if necessary to clear;
5. Remove from heat and cool to room temperature;
6. Filter with #3 Whatman filter paper (or whatever is handy that runs fast, such as pre-pleated paper for filtering the alcohol);
7. Add the 0.4 M Sodium Phosphate buffer (see formula below);
8. Add all but the last 100 mls of distilled water;
9. Add 1M HCl to adjust the pH to 7.4-7, check with pH paper;
10. Bring to final volume with deionized water.

Final Volume:	**1L**	**2L**	**3L**	**3.8L(1gal)**
Paraformaldehyde	40 g	80 g	120 g	152 g
water	750 mls	1.5 L	2.25 L	2.85 L
0.4 M buffer	250 mls	500 mls	750 mls	950 mls

Notes:

- Paraformaldehyde will never appear to be dissolving until the NaOH is added.
- Be patient, allow time for the NaOH to cause the paraformaldehyde to start dissolving before adding more.
- There is always a little colloidal "fluff" in unfiltered para.
- Don't weigh paraformaldehyde into plastic weigh boats - use a glass beaker to minimize static dispersion of paraformaldehyde dust.
- Wash out the filter paper in tap water before discarding, rinse everything having contacted the paraformaldehyde.

Sodium Phosphate Buffer, 0.4 M Stock, pH 7.4-7.6

	300 ml	**1 L**	**1.2 L**
$NaH_2PO_4 \cdot H_2O$ (sodium phosphate, monobasic, monohydrate)	3.18 g	10.6 g	12.72 g
K_2HPO_4 (potassium phosphate, dibasic, anhydrous)	16.8 g	56.0 g	67.2 g

Dissolve in ~90% of deionized water, adjust pH with 1 N HCl or 1 N NaOH, then bring to final volume. Phosphate salts of different hydration states can be used, but require adjustment for the differences in molecular weight.

1 N NaOH: 4 gm NaOH pellets dissolved to 100 ml with deionized water.
10 N NaOH: 40 gm NaOH pellets dissolved to 100 ml with deionized water.
1N HCl: 8.3 ml concentrated HCl is added to 91.7 ml deionized water.

Warning: NaOH generates heat while dissolving. Be prepared to cool the 10 N solution in a large beaker containing cold tap water.