

4 % PARAFORMALDEHYDE Fixative in 0.1 M. Sodium Phosphate Buffer, pH 7.2

An excellent all around fixative for normal histology, immunochemistry and autoradiology. Useful variations include addition of 0.1 - 0.25 % glutaraldehyde and/or 5 – 15 % saturated picric acid (v/v).

This fix is best used fresh; prepare it no more than 72 h in advance. Final molar concentrations of buffers are 23 mM monobasic and 77 mM dibasic.

	<u>For 1000 ml</u>	<u>For 4000 ml</u>
0.2M SODIUM PHOSPHATE BUFFER, pH 7.2	<u>500 ml</u>	<u>2000 ml</u>
sodium phosphate monobasic (NaH ₂ PO ₄ *H ₂ O, FW 137.99)	3.17 g	12.68 g
sodium phosphate dibasic (Na ₂ HPO ₄ , FW 141.96)	10.93 g	43.72 g
d ² H ₂ O	500 ml	2000 ml
PARAFORMALDEHYDE SOLUTION (8%)	<u>500 ml</u>	<u>2000 ml</u>
Paraformaldehyde	40 g	160 g
d ² H ₂ O	500 ml	2000 ml

Heat the paraformaldehyde solution to 60 - 65°C while stirring. Reduce heat and add 2 to 3 ml of 1.0 M NaOH, dropwise. After the solution clears, filter and add to an equal volume of 0.2M phosphate buffer.

Do not exceed the indicated temperature! Overheating of the paraformaldehyde for this (or any other paraformaldehyde based fixative) may adversely affect immunohistochemical procedures, both by increasing the background as well as decreasing specific staining.