Protocol for Making a 4% Formaldehyde Solution in PBS

Caution: Formaldehyde is toxic. Please read the MSDS before working with this chemical. Gloves and safety glasses should be worn and solutions made inside a fume hood.

The vast majority of IHC/ICC procedures employ fixation of tissues and cells using formaldehyde-based fixatives. The protocol below describes the technique for generating a 4% formaldehyde solution in PBS. The most effective fixative must be determined experimentally.

Please read the protocol in its entirety before starting.

### Procedure

1. For 1 L of 4% Formaldehyde, add 800 mL of 1X PBS to a glass beaker on a stir plate in a ventilated hood. Heat while stirring to approximately 60 °C. Take care that the solution does not boil.
2. Add 40 g of paraformaldehyde powder to the heated PBS solution.
3. The powder will not immediately dissolve into solution. Slowly raise the pH by adding 1 N NaOH dropwise from a pipette until the solution clears.
4. Once the paraformaldehyde is dissolved, the solution should be cooled and filtered.
5. Adjust the volume of the solution to 1 L with 1X PBS.
6. Recheck the pH, and adjust it with small amounts of dilute HCl to approximately 6.9.
7. The solution can be aliquoted and frozen or stored at 2 - 8 °C for up to one month. Please read the protocol in its entirety before starting.

### The difference between paraformaldehyde, formaldehyde, and formalin

Paraformaldehyde (chemical name is polyoxymethylene) is a powder of polymerized formaldehyde that by itself cannot fix tissues. To be usable as a tissue fixative, paraformaldehyde has to be dissolved in hot water to become a formaldehyde solution. Formalin is a saturated formaldehyde solution in water (37% by weight, 40% by volume) containing 10-15% methanol. Methanol is added to slow down the polymerization to formaldehyde, which reduces the fixing power of formalin. Formalin can also be made in an alcohol-free form from powdered paraformaldehyde.

### Reagents Required

<table>
<thead>
<tr>
<th>Reagents Required</th>
<th>Materials</th>
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<tbody>
<tr>
<td>Deionized H₂O</td>
<td>Filter units</td>
</tr>
<tr>
<td>HCl (Dilute)</td>
<td>Glassware and stir bar (dedicated for formaldehyde solution)</td>
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<td>NaOH (1 N)</td>
<td>Gloves and eye protection</td>
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<tr>
<td>Paraformaldehyde powder</td>
<td>Hot plate with magnetic stirrer</td>
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<tr>
<td>1X PBS: 0.137 M NaCl, 0.05 M NaH₂PO₄, pH 7.4</td>
<td>Thermometer</td>
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<td>Ventilated hood</td>
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</table>
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1. Digitally add 40 g of paraformaldehyde powder to 800 mL of 1x PBS heated to approximately 60 °C while stirring.
2. Add 1 N NaOH drop by drop until the powder dissolves.
3. Cool and filter with filter paper.
4. Adjust the volume to 1 L with 1x PBS.
5. Adjust the pH to 6.9 with dilute HCl.
6. Aliquot & freeze.