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https://protocols.io/view/4-formaldehyde-in-1m-pb-for-immuno-brains-1l-bivyke7w

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## .4M Phosphate buffer pH 7.4 (250mls)

21.7 g Na2 HPO4 (sodium phosphate dibasic-heptahydrous) [86.8 g]

~Make sure dibasic completely dissolves before adding monobasic!

2.6 g Na H2 PO4 (sodium phosphate monobasic-monohydrous) [10.4 a]

Dissolve in 200 ml of distilled H20 [800 mls]

pH to 7.4

Bring to 250ml volume with distilled H20

## In separate 1000 ml volumetric flask:[2000ml] 2.250 ml 0.4 M phosphate buffer (pH=7.4)

3.100 ml 37% formaldehyde [200mls]

2L1 4.Bring to 1L volume with distilled water (no need to re-pH)

5. Filter using side-arm flask (use glassware labeled 'paraform')

\*\*Please use gloves, protective eyeware and mask when working w/ formaldehyde.

Label and date 1L 4% Formaldehyde and put in plastic bottle marked

"4% FORM IMMUNO". Put in black KOOB fridge (by -80 freezer).

3 0.15 M Saline Pre-fixative Rinse (1 L) [2L]

1.Add 9.0 g NaCl to 800 mL dH<sub>2</sub>O. [18g]

2.Add ~1000 units of heparin per 1L saline [2mls]

3. Stir until dissolved (good for 1 week).

4. AdddH<sub>2</sub>Oto bring to volume.

5. Label and refrigerate