RECIPES FOR FIXATION OF TISSUE FOR ANTIBODY LABELING

Use paraformaldehyde fixation immediately following enucleation.

Primary fixative: 4% paraformaldehyde in 0.086M NaPO4, pH, 7.2

Stock sodium phosphate buffer (0.172 M NaPO4, pH 7.2)

Chemicals	500 ml	1000ml
NaH2PO4	3.41 g	6.82
Na2HPO4	8.66	17.32

1. To 90 ml of ddH2O at 80°C, add 8.0g of paraformaldehyde; stir until dissolved (in a fume hood). If needed, add a few drops of 1.0 N NaOH to help the paraformaldehyde go into solution. After it is completely dissolved, cool to room temperature.

2. Add 100 ml of stock sodium phosphate buffer (0.172M M NaPO4, pH, 7.2) to the paraformaldehyde solution.

3. Bring the final volume up to 200 ml with ddH20. This yields a 4% paraformaldehyde solution in 0.086M NaPO4 buffer, pH 7.2.

Alternate Fixation:

Use Sodium Cacodylate fixation for long-term storage of tissue or sections.

0.2 M Sodium Cacodylate Stock Buffer

For 500 mls add 21.4g of Na Cacodylate to 500 mls ddH_2O. Wear gloves.

4% Paraformaldehyde in 0.1M Sodium Cacodylate Buffer

For 1 Liter, add 40g paraformaldehyde to 500 mls 0.2 M Na Cacodylate buffer. On a hot plate, in the hood, heat mixture until paraformaldehyde dissolves and solution clears.
Add about 400 mls of ddH_20 and let solution cool.
Adjust the pH to 7.4 using 1N HCl.
Pour solution into graduated cylinder and bring volume up to 1 liter with ddH20.