

Making FIXATIVE for In Situ and Antibody Staining

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4% Paraformaldehyde/1XPBS Fix for RNA In Situ Hybridization

1. Make RNase-free glassware and stir bars by adding a stir bar to a 500 ml bottle, adding 10 ml of 10N NaOH, filling bottle half full with distilled water, and swishing well to coat inside of bottle.
2. Carefully dump out contents of bottle and rinse well with more distilled water. Rinse stir bar and reserve on a clean paper towel on the side.
3. Fill bottle with ~400 ml of ultrapure RNase free water and microwave on high for 4 minutes until water is boiling.
4. Meanwhile, weigh out 20 g of paraformaldehyde (use gloves).
5. Place boiled water on a stir plate in a hood, add RNase free stir bar. Carefully add 20 g paraformaldehyde powder.
6. Add 50 ul of 10N NaOH.
7. Stir until paraformaldehyde is completely dissolved and solution has cooled to ~40-50°C.
8. Add 50 ml 10X Ca⁺² and Mg⁺² free PBS.
9. Using disposable RNase free volumetric cylinder or disposable 50 ml tube, measure out fixative into a sterile filtration apparatus. If volume is less than 500 ml, add ultrapure RNase free water to a final volume of 500 ml. Check that pH=7.0!
10. Turn on vacuum and filter fixative. Store at 4°C and use within 2-3 days.

4% Paraformaldehyde/1XPBS Fix for Antibody Staining-500 ml

1. Measure out ~400 ml distilled water into glass bottle and microwave on high for 4 minutes until water is boiling
2. Meanwhile, weigh out 20 g of paraformaldehyde (use gloves).
3. Place boiled water on a stir plate in a hood, add stir bar, and carefully add 20 g of paraformaldehyde powder.
4. Add 50 ul of 10N NaOH
5. Stir until paraformaldehyde is completely dissolved and solution has cooled to ~40-50°C.
6. Add 50 ml 10X Ca⁺² and Mg⁺² free PBS.
7. Bring final volume of fixative to 500 ml and check that pH=7.0! Sterile filter and store at 4°C and use within one week.

4% Paraformaldehyde/1XPBS Fix for Antibody Staining-100 ml

Same as above except:

1. Use ~80 ml of distilled water.
2. Weigh out 4 g of paraformaldehyde(use gloves).
3. Add 10 ul of 10N NaOH.
4. Add 10 ml 10X Ca⁺² and Mg⁺² free PBS.
5. Bring volume to 100 ml final.