**4% Paraformaldehyde Fixative (0.1 M) Recipe**

**4% PFA Solution – pH 7.4 400 mL 800 mL**

* *Disodium phosphate (Na2HPO4) 4.36 g 8.72 g*
* *Monosodium phosphate (NaH2PO4) 1.28 g 2.56 g*
* *Paraformaldehyde powder 16.0 g 32.0 g*
* *MilliQ water (H2O) to 400 mL 800 g*
* *Sodium hydroxide (NaOH) as needed as needed*
* *Hydrogen chloride (HCl) as needed as needed*

**Materials needed**

1 L / 2 L beaker

500 mL / 1 L bottle

Filter paper

Funnel

Gloves

Hot plate

Magnetic stir bar & retriever

pH meter

Thermometer

*Note: Only use items marked with “F” when making fixative. Wear gloves!*

**Protocol**

1. Add 350 mL (or 750 mL) of water, magnetic stir bar, and thermometer to a 1 L (or 2 L) beaker.
2. Using a hot plate in the fume hood, heat water to ~68°C.
   1. Make sure temperature does not exceed 70°C.
3. Turn off heat element and remove thermometer.
4. Add paraformaldehyde powder over 10 minutes. Stir vigorously to dissolve.
5. Add drops of NaOH until the solution is clear when settled.
6. Add Na2HPO4 and NaH2PO4 to solution.
7. Cool solution to room temperature before adjusting the pH with HCL. Final pH should be 7.4 at room temperature.
8. Add water up to appropriate final volume.
9. Filter into a 500 mL (or 1 L) bottle and store in the fridge at 4°C.