

## 4% Paraformaldehyde Fixative (0.1 M) Recipe

4% PFA Solution – pH 7.4	400 mL	800 mL
– Disodium phosphate ( $\text{Na}_2\text{HPO}_4$ )	4.36 g	8.72 g
– Monosodium phosphate ( $\text{NaH}_2\text{PO}_4$ )	1.28 g	2.56 g
– Paraformaldehyde powder	16.0 g	32.0 g
– MilliQ water ( $\text{H}_2\text{O}$ ) to	400 mL	800 g
– Sodium hydroxide ( $\text{NaOH}$ )	as needed	as needed
– Hydrogen chloride ( $\text{HCl}$ )	as needed	as needed

### Materials needed

1 L / 2 L beaker	Hot plate
500 mL / 1 L bottle	Magnetic stir bar & retriever
Filter paper	pH meter
Funnel	Thermometer
Gloves	

*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  $\sim 68^\circ\text{C}$ .
  - a. Make sure temperature does not exceed  $70^\circ\text{C}$ .
3. Turn off heat element and remove thermometer.
4. Add paraformaldehyde powder over 10 minutes. Stir vigorously to dissolve.
5. Add drops of  $\text{NaOH}$  until the solution is clear when settled.
6. Add  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$  to solution.
7. Cool solution to room temperature before adjusting the pH with  $\text{HCL}$ . 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  $4^\circ\text{C}$ .