**4% Paraformaldehyde (PFA) in PBS Protocol:**

*Note: Protocol based on 1L final volume of 4% paraformaldehyde (PFA)*

1. In a chemical fume hood, take 500mL of dH2O (half of total desired volume) and pour in beaker. Add 40g of solid PFA (8% of dH2O volume). Add magnetic stir bar, cover with parafilm, and heat on magnetic hot plate at 60 °C. Make sure that solution does not exceed 60 °C.

2. After solution is heated to 60 °C and PFA is partially dissolved (dependent on hot plate, approximately 20 min to 1 hour), add two pellets of NaOH to facilitate dissolution. Wait ~30 min and if PFA does not dissolve, add one pellet of NaOH every 15 min until precipitate is fully dissolved.

3. Once solution is clear—indicating full dissolution of PFA in dH2O—remove from hot plate and filter through funnel and qualitative filter into a graduated cylinder (the final volume should be greater than 500mL because addition of PFA should have altered total volume). This is 8% PFA in dH2O.

4. Make up the same volume of 2x PBS that you measured 8% PFA in step 3 (i.e. 550mL 2x PBS if you ended up with 550mL of 8% PFA in step 3, etc.)

5. Mix solutions from step 3 (8% PFA) and step 4 (2x PBS) 1:1 to make a solution of 4% PFA in 1x PBS. (Note that the final volume of 4% PFA should come out to more than 1L because of the change in volume of solution when PFA was dissolved in dH2O)

6. Use a pH strip to measure pH of solution. Solution should be approximately pH = 7.2. If it is more acidic, add one pellet of NaOH at a time while stirring with stir bar until proper pH is reached. If it is more basic, do the same thing but with 20uL of HCl at a time instead.

7. Store at 4 °C for a week or at -20 °C in aliquots for a month.