**4% PFA: Rebecca Notes**

| 4% PFA | DI Water | PFA Powder | 10x PBS |
| --- | --- | --- | --- |
| 1L | 900 mL | 40 g | 100 mL |
| 500 mL | 450 mL | 20 g | 50 mL |
| 250 mL | 225 mL | 10 g | 25 mL |

**Paraformaldehyde** is a dangerous chemical and caution must be exercised when working with it. Solutions and vapors are flammable, are possibly carcinogenic, and are irritating to the eyes, skin, and lungs. A **lab coat**, eye protection, and gloves are required when handling paraformaldehyde. A **fume hood** is required for the preparation and usage of paraformaldehyde solutions. Latex gloves should provide adequate protection when skin contact with paraformaldehyde solutions is minimal, such as while fixing tissue. **Nitrile gloves** are recommended for handling saturated speciminies or if dipping hands in solutions.

Paraformaldehyde does not readily dissolve in solution at room temperature and therefore must be heated. Best results are achieved by first heating the paraformaldehyde with only water at first and then adding phosphate buffered solution (PBS)

1. To prepare a 4% phosphate-buffered solution (final volume 1L) place a magnetic stirrer/hotplate in fume hood.
2. Place a temperature probe or thermometer into a suitable container such as a conical flask or beaker along with a magnetic stir bar.
3. Pour measured amount of deionized water (900 mL) and then add (40 g) of paraformaldehyde powder and heat to 55 C. It is important to avoid overheating the solution (keep <60 C).
   1. Use weighboat, spatula, scale in hood (some items located in drawers across from hood) to measure out powder
4. Once the solution is dissolved (the time it takes for this is dependent on the hot plate, approximately 15 min to 30 min) add 0.25 g (~2 pellets), of sodium hydroxide (NaOH) and leave on the hot plate/stirrer until the solution becomes clear (<5 min).
   1. If the solution is not fully clear, add another NaOH pellet.
   2. These pellets are found on shelf across from the hood
   3. If not fully clear, add another pellet and stir again
5. Then add (100 mL) of 10x PBS and leave on the stirrer for an additional 30 seconds.
6. Remove from heat and allow to cool. Once the solution has reached room temperature, filter using filter paper and a funnel into your 1L bottle.
7. Use a pH strip to measure the pH of the solution. Solution should be approximately pH=7.2.
   1. It will typically be basic at this point and you will need to add HCl.
   2. Be very careful as this is a highly corrosive chemical.
   3. Start with 10 uL and test pH again. Continue doing this until the desired pH is reached.
8. Clean up, label bottle with PFA (red tape); store at 4C
9. **General notes:** 
   1. If you need to make more basic = add NaOH pellet
   2. If you need to make more acidic = add HCl (in the flammable cabinet).
      1. .5 ml at a time (w/ pipette)