## 1. Prepare 1N NaOH and 1N HCl for adjusting pH later on (can be made in advance).

- a. To make a 1N solution of NaOH, dissolve 10 grams of NaOH in 250 mL of sterile water
- b. To make a 1N solution of HCl, get 8.3 ml of 37% HCl (equal to 12N) and slowly add to 91.7 ml of sterile water. Remember to add acid to sterile water in this order. If you add water to acid, you are risking getting burned. Acid + water = an exothermic reaction. High temperature of the solution might make the glass break. Be careful and prepare under the hood and ask for help if needed!
- 2. Take 700 mL of 1X PBS.
- 3. Add 40 g of Paraformaldehyde powder to 1X PBS (under the hood).
- 4. Stir the mixture at  $60^{\circ}$ C (DO NOT Boil) for  $\sim 30$  min.
- 5. After 30 min, add 1N NaOH drop by drop until a clear solution is formed.
  - a. Why? PFA powder does not dissolve instantly, you need to raise the pH of the mixture.
- 6. Adjust pH with 1N NaOH and 1N HCl to 6.9 7.4 if needed.
- 7. Adjust the volume to 1L with 1X PBS.