

Preparation of 4% Paraformaldehyde (PFA)

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Introduction

This protocol describes the preparation 4% paraformaldehyde (PFA).

Definitions

HCL: hydrochloric acid

NaOH: sodium hydroxide

PBS: phosphate buffered saline

Reagents and Materials

- 1-liter 1X PBS
Lot #: _____
Manufacturer: Gibco
- PFA powder
Lot#: _____
Amount Weighed out: _____
Source: Fisher Scientific
- 1N NaOH
Manufacture date: _____
Lot#: _____
Manufacturer: In house
- pH 4.00 Standard Buffer
Lot#: _____
Manufacturer: Fisher Scientific
- pH 7.00 Standard Buffer
Lot#: _____
Manufacturer: Fisher Scientific
- pH 10.00 Standard Buffer
Lot#: _____
Manufacturer: Fisher Scientific
- Dilute HCL
Manufacture date: _____
Lot#: _____
Manufacturer: In house

- Millipore 0.22 μm GSWP Nitrocellulose Membrane (aqueous filter)
Lot#: _____
Manufacturer: Millipore/Fisher Scientific

Instrumentation

Fisher Accumant Basic AB15 Plus pH Meter with pH probe
Probe SN: _____
Manufacturer: Fisher Scientific

Heated Stir Plate
Manufacturer: Fisher Scientific

Glass Thermometer
Manufacturer: Fisher Scientific

Protocol

Note: Steps 1 through 5 have to be done in a chemical fume hood

1. Add 800 mL of 1X PBS into clean, dry, 1.5 L glass beaker with clean magnetic stir bar
2. Heat solution while stirring to approximately 60 °C; take care that the solution does not boil, use glass thermometer to monitor
3. Add 40 g of PFA powder to heated PBS; powder will not immediately dissolve into solution
4. Slowly raise the pH by adding 1 N NaOH dropwise until the solution clears
5. Once the solution clears, remove from heat and allow to cool to room temperature
6. Adjust the volume of the solution to 1 L with 1X PBS
7. Standardize pH probe using three pH standard buffers, rinsing probe with HPLC grade water between samples, wipe dry with Kim Wipe;
Slope _____
8. Rinse and dry pH probe, measure pH of solution
9. Adjust pH down to 6.9 with small amounts of dilute HCl, dropwise;
Final pH _____
10. Filter using vacuum flask through aqueous filter paper
11. Transfer buffer to a clean, dry storage bottle; Label _____
12. Store at 4 °C