



Title: Paraformaldehyde Fixation Buffer (PFA)

No: RTLP-BSS-15

Location:
Old CCRC Tripp Lab

Approval Date:
14 July 2005

Supersedes Date:

Materials:

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|--------------------|-------------------------|------------------|---------------------|
| •Lab coat | •Dulbecco's Phosphate | •Stir/heat plate | •Pipettes |
| •Gloves | Buffered Saline (PBS) | •Thermometer | •Pipetteman |
| •Mask | •Small transfer pipette | •Microwave | •Pipette Aid |
| • Paraformaldehyde | •1N NaOH | •pH strips | •Pipetteman tips |
| | | •stir bar | •Analytical Balance |

Procedure: This protocol makes 500mL of a 2% solution. Adjust according to your needs. Keep in mind, PFA is stable for only two weeks.

NOTE: Paraformaldehyde is a neurotoxin and should be treated as a carcinogen. Wear **MASK** and **GLOVES** when preparing this solution!! Fumes resulting from heating should **NOT** be inhaled, therefore preparation must be performed in the laminar flow hood. Mixture should **never** go above 60°C!

1. Heat 100mL of Dulbecco's Phosphate Buffered Saline (D-PBS) to ~55°C in the microwave. Do not boil. Place heated D-PBS onto heated stir plate- do not allow heat to rise above 60°C. This will ruin the paraformaldehyde.
2. Weigh out 10g of paraformaldehyde using the analytical balance (glass encased) to prevent spread of fine powder. (Be sure to clean up any powder spills with dH₂O followed by ETOH).
3. Add paraformaldehyde to D-PBS and stir with heat. Again, do not allow heat to rise above 60°C. This will ruin the paraformaldehyde.
4. Using a small transfer pipette, add one drop of 1N NaOH to the cloudy solution. Once paraformaldehyde has gone into solution, the mixture will become clear (this should occur within 2 minutes). BE PATIENT!! If paraformaldehyde powder is still

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visible, add one more drop and wait 2 minutes. Continue until paraformaldehyde goes into solution.

5. Use pH strips to determine pH of paraformaldehyde, which should be 7.2-7.4. If pH is higher, it is NOT possible to bring down by using HCL. You must start from scratch. Typically, the cause of high pH is overuse of NaOH- again, BE PATIENT!
6. Let paraformaldehyde cool, then bring up to 500mL with D-PBS.
7. Pour solution into a light sensitive (brown) or foil covered glass bottle and store at 4°C. This PFA can be used for up to two weeks, at which time it will begin to break down and is therefore useless. Discard remaining waste into designated hazardous waste container.