

Fixing Cells with Paraformaldehyde (PFA) for Flow Cytometry

Preparation of Working Solutions:

- Dilute only the amount of PFA you will need per experiment to 4% PFA from the 16% stock with PBS.
- Store the undiluted stock at -20 degrees until needed (open stocks should only be kept for one month)
- Add an equal volume of the 4% stock to samples for a final concentration 2% PFA.
 - Fixation can be done from 0.5-2%.
- Prepare your cells for flow cytometry (block, stain, wash etc...)
- Fix cells on ice for 15-30 minutes on ice, and then wash twice with PBS.
 - Verify the length of time required to fix the sample type... special considerations may be required for virally infected samples etc.

Notes:

- It is recommended that data is recorded as soon as possible after staining and fixation is complete, however samples can be left for a few days if needed.
- Be careful when staining tandem dyes like PE-Cy7 and APC-Cy7, the Cy7 moiety is damaged by fixation.
 - Use the *minimum* amount of time possible to achieve adequate fixation for your sample type and BSL.
- Samples should never be left in PFA overnight. This dramatically increases the amount of autofluorescence your samples.
- Always date your working solutions, diluted PFA (2-4% solutions) are only good for 1 week.

Preparation of 2% Paraformaldehyde from Powder

- Allow paraformaldehyde (PFA) powder to come to room temperature (Stored in refrigerator).
- Weigh 10.0 g PFA in fume hood.
- Flush container with Argon or Nitrogen to prevent air decomposition of p-formaldehyde.
- Dissolve in 475 ml distilled H₂O in 60-70° C water bath on hot plate in fume hood. Do not allow water bath to go over 70°C (formaldehyde will vaporize!).
- While dissolving, label 100 X 4 ml and 7 X 12 ml tubes (or other combinations of useful aliquots) with the concentration and date (2%).
- After @ 1 hr add 1 or 2 drops of 5M NaOH. Cloudy suspension will then turn clear.
- Allow to cool to room temperature (@ 2 hours).
- Add 25 ml 20X PBS ([see recipe](#)) and adjust pH to 7.3.
- Filter, aliquot into tubes and freeze. Aliquots good for at least 5 years.

Working Diltution:

- Thawed aliquots are stable at 4° C for up to 2 weeks.
Dilute 1 part 2% PFA to 3 parts cells in PBS (eg 60 µl + 180 µl to yield 0.5% final concentration).

References:

1. Becton Dickinson Immunocytometry Systems Source Book (1989) 2.10
2. Lanier, L.L., and Warner, N.L. (1981) Paraformaldehyde Fixation of Hematopoietic Cells for Quantitative Flow Cytometry (FACS) Analysis. *Journal of Immunological Methods* 47, 25
3. C.A. Williams, M.W. Chase, Stabilizes cell membranes and preserves cell morphology. *Methods Immunol. Immunochem.* New York **5**, (1976)

Reagent Suppliers:

Canemco: www.canemco.ca PFA: # 0173 16% solution, 10 mL Ampoules

Aldrich #30525-89-4
Sigma P-6148