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 H_2O in 60-70º C water bath on hot plate in fume hood. Do not allow water bath to go over 70ºC (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 4º 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