Becton Dickinson Procedures

Paraformaldehyde Fixation of Fluorescent Stained Hematopoietic Cells

This procedure is used to preserve hematopoietic cells stained with fluorochrome conjugated monoclonal antibodies for subsequent analysis by fluorescence microscopy or flow cytometry. The fixed cells may be stored for at least one week at 4° C in the dark. This method of fixation does not significantly alter the volume, light scatter, or fluorescence properties of mouse, rat or human lymphoid cells, or transformed cell lines. [1]

Cells:

1. Mouse, rat or human cells stained by a direct or indirect immunofluorescence technique (see Becton Dickinson Procedures on Direct Immunofluorescence Staining of Cell Surfaces, Source Book Section 2.4, and Indirect Immunofluorescence Staining of Cell Surfaces, Source Book Section 2.5).

Reagents:

1. Phosphate-Buffered Saline (PBS) Solutions:

   25X Stock Solution

   Dissolve in 1 liter of distilled water:

   188 g K_2HPO_4
   33 g $NaH_2PO_4 \cdot H_2O$
   180 g NaCl

   1X Working Solution

   Add 40 ml 25X Stock Solution to 960 ml of distilled water. Add 1 g sodium azide. Mix well.

WARNING: Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be diluted with running water before being discarded. These conditions are recommended to avoid deposits in plumbing where explosive conditions may develop.

2. 0.85% Saline:

   Dissolve 0.85 g NaCl in 100 ml of distilled water.

3. 2% Paraformaldehyde Solution:

   Add 2 g paraformaldehyde (Eastman Kodak) to 100 ml 0.85% saline. Heat to 70° C in fume hood until the paraformaldehyde goes into solution. Allow the solution to cool to room temperature. Adjust to pH 7.4 using 0.1 M NaOH or 0.1 M HCl as needed. Store at 4° C.

Equipment:

1. Refrigerated centrifuge fitted with swinging bucket rotor (4° C)
2. Ice bucket

Procedure: *

1. Following the last incubation with antibody or fluorescent second-step reagent, centrifuge the cells at 400 x g for 10 minutes. Remove the supernatant.
2. Add 1 ml of cold 1X PBS per 10^6 stained cells. Gently vortex the cells and centrifuge at 400 x g for 10 minutes. Remove the supernatant.
3. Repeat Step 2.
4. Add 0.3 to 1.0 ml of cold paraformaldehyde solution to the pellet. **Vortex** the cell suspension **immediately.**
5. Store the fixed cells at 4° C in the dark. These cells may be stored for at least one week prior to FACS analysis.

* Note: Cells may also be fixed following staining in microtiter plates using these modifications:

1. Following the last staining step, centrifuge the cells (~ 10^6/well) in microtiter plates at 250 x g for 5 minutes. Carefully remove the supernatants.
2. Add 100 µl PBS to each well. Centrifuge at 250 x g for 5 minutes. Carefully remove the supernatants.
3. Repeat Step 2.
4. Add 100 µl of cold 2% paraformaldehyde solution to each well and **mix thoroughly at once.**

References:

[1] Lanier, L.L., and Warner, N.L. (1981) Paraformaldehyde Fixation of Hematopoietic Cells for Quantitative Flow Cytometry (FACS) Analysis. *J. Immunol. Meth. 47*, 25 - 30

Becton Dickinson Monoclonal Center, Inc.
2375 Garcia Avenue
Mountain View, California 94043

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