PHOENIX FLOW SYSTEMS

Cell Permeabilization Procedure for Staining of Intracellular Antigens

In the past there have been issues with the proper permeabilization of our TdT control cells using some of the commericial available permeabilization solutions. This recipe works well for all cell types and our CRISP control cells. If you don't want to make it yourself, we will be happy to sell you a bottle for a few bucks.

Reagents:

IFA Permeabilization Media (PFS cat# IFA1001) (1X PBS, pH 7.4, 0.1% Triton X-100, 4% FBS)
Note: Although our IFA reagent contains sodium azide as a preservative, care should be taken to avoid contamination of the stock solution.
1% paraformaldehyde (prepared fresh from 10% stock)

Procedure: **Precipitate may appear in bottom of IFA Media. This is normal upon refrigeration. Remove IFA Media from refrigerator and bring to room temperature prior to use to dissolve precipitate. <u>Do not</u> use until all particulate material is in solution.**

1. Label tubes according to flow cytometry worksheet.
2. Add 100ul (1 e6 cells) of well mixed CRISP Control or sample cells to be tested to each tube. Keep tubes on ice.
3. Add 2ml of 1% paraformaldehyde/PBS. Cap and vortex. Incubate for 15 minutes on ice.
4. Centrifuge all tubes at 300 X g for 5 minutes.
5. Carefully discard all supernatant.
6. Add 2 mls of IFA media to each tube. Cap, vortex, and incubate for 3-5 minutes on ice.
7. Centrifuge all tubes at 400 X g for 10 minutes.
8. Carefully discard all supernatant.
9. Add appropriate antibody to the appropriately labeled tubes.
10. Cap all tubes, gently vortex.
11. Incubate at room temperature in the dark for 1 hour.
12. Add 2 mls of IFA media to each tube. Cap and gently vortex.
13. Centrifuge all tubes at 400 X g for 5 minutes.
14. Carefully discard all supernatant.
15. Resupend in PBS if acquiring within 1 hour. Otherwise, resuspend in 1% paraformaldehyde/PBS.

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