Flow Cytometry

BETHYL LABORATORIES, INC

Flow Cytometry Protocol for Indirect Intracellular Staining of Cultured Cells Grown in Suspension.

Reagents Required:

16% paraformaldehyde (PFA) (Electron Microsopy Sciences RT 15710)
90% Methanol (ice cold)
PBS (Phosphate Buffered Saline pH 7.2)
- 11.6 g sodium chloride
- 10 ml 1 M phosphate buffer
- 0.26 ml 4 M sodium hydroxide
- DI H_2O to 1 liter

ICSB (IntraCellular Staining Buffer)
- 495 ml PBS
- 5 ml FBS (fetal bovine serum)
- 0.36 ml 9% sodium azide

12 x 75 FACS tubes (BD Falcon #352054)

Cell Fixation and Permeabilization:

1. For cells grown in suspension, add fresh 16% PFA to achieve a final concentration of 1.54% directly to the cells in media (1-2 x 10^6 cell/ml).
2. Fix cells at room temperature (RT) for 10 minutes.
3. Incubate and fix on ice for an additional 30 minutes.
4. Spin fixed cells at 250 x g for 5 minutes at 20°C.
5. Pour off media + PFA into dedicated PFA waste containier.
6. Permeabilize cells by resuspending in ice cold 90% methanol to a final cell concentration of 1 X 10^6 cells/ml.
7. Cells may be stored at -70° to -20°C for up to a month.

Intracellular Staining:

1. Aliquot 1 ml (1 x 10^6 cells) of cells in methanol for each tube/sample.
2. Spin at 250 x g for 5 minutes at 20°C, brake set to slow (perform all subsequent spins at these conditions)
3. Pour off methanol.
4. Resuspend cells in 1 ml ICSB for wash.
5. Pour off wash and blot tube on paper towels.
6. Resuspend cells in 50 mcl of primary antibody at desired dilution.
7. Incubate at RT for 1 hour.
8. Add 1 ml of ICSB.
9. Spin.
10. Pour off and blot tube.
11. Resuspend in 100 mcl of conjugated secondary antibody at desired dilution.
12. Incubate 30 minutes at RT in dark.
13. Add 1 ml ICSB for 2nd wash.
14. Spin, pour off, and blot tube.
15. Resuspend in 100 mcl ICSB.
16. Analyze on a flow cytometer.