Flow Cytometry Protocol

Antibody Conjugation Protocol

ID#RM02

Molecular Probes' Amine-Reactive Probes Guide
(https://tools.thermofisher.com/content/sfs/manuals/mp00143.pdf)

Note: We have used Alexa 488 and Alexa 594 succinimidyl esters to label primary antibodies. Due to the small volumes and relatively low concentrations at which primary antibodies tend to be provided, we have scaled down the protocol. We have had success labeling full vials of antibodies at a time (generally about 0.1 mg in 200 uL). We strongly recommend labeling five or more antibodies at a time due to the difficulty in massing the dyes and their instability in DMSO.

Alterations that we have made to the protocol include:

1. Labeling at below optimal concentrations of antibody (we often perform the reaction directly in the vial of primary antibody from the vendor)
2. Increasing the ratio of dye to protein (label 5 – 8 tubes of primary antibody per 1 mg of dye)
3. Dialyzing with 2 L of PBS in the dark at room temperature for 2 h rather than using a column to purify the conjugated antibody (We have used 20,000 MWCO Slide-A-Lyzer MINI Dialysis Units from Thermo Scientific, catalog #69590, with Slide-A-Lyzer MINI Dialysis Floats, catalog #69588)

Brief protocol:

1. Add 30 uL of 0.75 M sodium bicarbonate, pH 8.3, to 200 uL of primary antibody (label 5-8 tubes of primary antibody to minimize waste of dye, as it loses stability when dissolved)

2. Dissolve the 1 mg vial of succinimidyl ester dye in 100 uL DMSO

3. Slowly add 12.5 – 20 uL of dye to each vial of primary antibody while vortexing the solution

4. Shake the tubes for 1 h at room temperature (we have used a bacterial shaker at 200 RPM)

5. Add 20 uL of freshly prepared 1.5 M hydroxylamine, pH 8.5 to each tube and shake for 1 h at room temperature

6. Dialyze using 2 L of PBS in the dark at room temperature for 2 h

We have then stored at 4^{o}C without adding preservatives, but adding BSA and/or sodium azide is recommended in the original protocol.