3-Color Intracellular Protocol

Reagents

HBSS
Saponin
PBS, Ca^{++}, Mg^{++}-free
Hamster anti-Mouse Bcl-2 mAb
Armenian Hamster IgG Isotype Control
Goat IgG serum
Rat anti-Mouse CD4-PE
Rat anti-Mouse CD8-PE/Cy5
Goat anti-Hamster FITC (GAH-FITC)
HBSS+ (HBSS + 1%BSA)

Permeabilization Soln.
0.15g saponin/50mls PBS

Wash & Stain Soln.
0.15g saponin/500mls PBS

Fresh Paraformaldehyde (PFA) **Fix:**
0.2g formaldehyde/10mls PBS; heat to clarify and filter, 0.45um

Procedure

1. Label $2X10^6$ single cells with directly conjugated antibodies by standard cell surface labeling protocol. Wash cells 2X in HBSS+ and 1X in HBSS.
2. Re-suspend cells in 200ul PBS and vortex. Add 200ul **FIX** and vortex. Incubate 10' on ice.
3. Wash cells with 2mls PBS. Centrifuge cells (~1,500 rpm, 10', 4^0C). Wash with 1ml Wash & Stain Soln. Pellet cells
4. Re-suspend cells in 400ul Permeabilization Soln. and incubate 30' on ice.
5. Wash 2X with 1ml Wash & Stain Soln. Prepare antibody mix and add 100ul to each tube. Incubate 30' on ice.

IgG Control primary mAb
2.0ul (1.0ug) Hamster IgG
88.0ul Wash & Stain Soln.
10.0ul Goat serum
100.0ul

Bcl-2 priamry mAb
2.0ul (1.0ug) Hamster anti-Mouse *Bcl-2* mAb
88.0ul Wash & Stain Soln.
10.0ul Goat serum
100.0ul

6. Wash 3X in 1ml Wash & Stain Soln. Prepare secondary antibody mix and add 100ul to each tube. Incubate 30' on ice

GAH-FITC secondary mAb
2.0ul (1.0ug) Goat anti-Hamster IgG-FITC
88.0ul Wash & Stain Soln.
10.0ul Goat serum
100.0ul

7. Wash 3X with 1ml Wash & Stain Soln. Re-suspend cells in 100ul Wash & Stain Soln. and add 100ul **FIX** and vortex. Provide proper controls and analyze by flow cytometry.