

Cell Surface Labeling Protocol

Reagents

PBS, Ca⁺⁺, Mg⁺⁺ - free

2%FBS/media

1% Formaldehyde (aka PFA)/PBS (heated to clarify and 0.45um filtered)

Direct Labeling

Primary fluorochrome-conjugated antibody Exp.: Rat anti-Mouse CD4-PE

Isotype Control (-) Exp.: Rat IgG-FITC

Indirect Labeling

Primary unconjugated antibody Exp.: Rat (IgG) anti-Mouse CD3

Secondary fluorochrome-conjugated antibody Exp.: Donkey anti-Rat FITC

Isotype Control (-) Exp.: Rat IgG-FITC

Fluorochrome Control (-) Exp.: Donkey anti-Rat FITC

Procedure

1. Prepare a single cell suspension and wash 1×10^6 cells in cold PBS. Wash cells 1X in 2%FBS/media. Centrifuge cells to pellet (~1,200 rpm, 5', 4⁰C) and aspirate supernatant.
2. Re-suspend cells in a final volume of 50ul containing the primary antibody at the correct titer and 2%FBS/media. Typically 1ug/ 10^6 cells. Incubate (4⁰C or room temp) 45' – 60'
3. Wash cells 2X in 2%FBS/media.
4. For a directly conjugated antibody go to step #5. For indirect labeling re-suspend cells in a final volume of 50ul containing the secondary antibody and 2%FBS/media. Incubate (4⁰C or RT) 45' – 60'
5. Re-suspend cells at a final cell density of $1-2 \times 10^6$ cells/ml in 1% PFA. Provide the proper isotype and single color controls for compensation.