Cell Surface Staining Protocol

Cell Harvesting

Spin down cell suspension at 1000 RPM for 5 minutes and decant supernatant. Resuspend the pellet in 1X PBS. Count the cells with a hemocytometer. Add the total desired number of cells to a flow tube (generally 0.5-1 x 10e6 per sample). Wash the cells by adding ~1 ml (or more if many samples) of 1X PBS to the flow tube. Spin down cell suspension at 1000 RPM for 5 minutes and decant supernatant. Gently tap the tube to loosen the cell pellet. Add an appropriate volume of staining buffer (generally 50 ul per 1 x 10e6 cells). Add 1 x 10e6 cells (generally 50 ul) to the desired number of flow tubes.

Staining

-Primary

Add the full amount of antibody to 50 ul of staining buffer and add this to the 50 ul of cell suspension, pipetting up and down to mix.

a. Fluorescent labeled primary antibody: Incubate on ice for 30-60 min; protect from light during incubation.

b. Unlabeled primary antibody: Incubate on ice for 30-60 min.

-Secondary (unlabeled primary only)

Add ~1 ml of staining buffer and spin down cells at 1000 RPM for 5 min. Decant supernatant and wash cells twice with 1-2 mls of staining buffer. Resuspend each cell pellet with 100 ul of secondary antibody solution. Incubate on ice for 30 min; protect from light during incubation.

Final Wash and Data Acquisition

Wash cells twice with 1-2 ml of staining buffer. After the final decanting, resuspend stained cells in an appropriate amount of staining buffer. Acquire data on a flow cytometer following manufacturer's recommendations.

Intracellular Staining Protocol

Cell Harvesting

Spin down cell suspension at 1000 RPM for 5 minutes and decant supernatant. Resuspend the pellet in 1X PBS. Count the cells with a hemocytometer. Add the total desired number of cells to a flow tube (generally 0.5-1 x 10e6 per sample). Wash the cells by adding ~1 ml (or more if many samples) of 1X PBS to the flow tube. Spin down cell suspension at 1000 RPM for 5 minutes and decant supernatant. Gently tap the tube to loosen the cell pellet.

Fixation and Permeabilization

Resuspend the cell pellet with the desired volume of 1X fixation buffer (generally 50 µl per 1 x 10e6 cells). Incubate at room temperature in the dark for 30 min, then spin down cells at 1000 RPM for 5 min and decant supernatant. Resuspend the cell pellet with 1-2 mls of 1X permeabilization buffer and spin down cells at 1000 RPM for 5 min. Decant supernatant and repeat wash step. Dump off supernatant and add 50 ul of 1X permeabilization per 10e6 cells in the pellet. Pipette up and down to evenly suspend cells. Add 50 ul of cell suspension to the desired number of flow tubes.

Staining

-Primary

Add the full amount of antibody to 50 ul of permeabilization buffer and add this to the 50 ul of cell suspension, pipetting up and down to mix.

a. Fluorescent labeled primary antibody: Incubate at room temperature for 30-60 min; protect from light during incubation.

b. Unlabeled primary antibody: Incubate at room temperature for 30-60 min.

-Secondary (unlabeled primary only)

Add ~1 ml of permeabilization buffer and spin down cells at 1000 RPM for 5 min. Decant supernatant and wash cells twice with 1-2 mls of permeabilization buffer. Resuspend each cell pellet with 100 ul of secondary antibody in permeabilization buffer. Incubate at room temperature for 30 min; protect from light during incubation.

Final Wash and Data Acquisition

Wash cells twice with 1-2 ml of staining buffer. After the final decanting, resuspend stained cells in an appropriate amount of staining buffer. Acquire data on a flow cytometer following manufacturer's recommendations.