

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 $0.5-1 \times 10^6$ 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 μ l per 1×10^6 cells). Add 1×10^6 cells (generally 50 μ l) to the desired number of flow tubes.

Staining

-Primary

Add the full amount of antibody to 50 μ l of staining buffer and add this to the 50 μ l 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 μ l 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 $0.5-1 \times 10^6$ 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 μ l per 1 x 10⁶ 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 μ l of 1X permeabilization per 10⁶ cells in the pellet. Pipette up and down to evenly suspend cells. Add 50 μ l of cell suspension to the desired number of flow tubes.

Staining

-Primary

Add the full amount of antibody to 50 μ l of permeabilization buffer and add this to the 50 μ l 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 μ l 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.