

## 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 10<sup>6</sup> 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 10<sup>6</sup> cells). Add 1 x 10<sup>6</sup> 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 10<sup>6</sup> 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  $\mu$ l per 1 x 10<sup>6</sup> 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  $\mu$ l of 1X permeabilization per 10<sup>6</sup> cells in the pellet. Pipette up and down to evenly suspend cells. Add 50  $\mu$ l of cell suspension to the desired number of flow tubes.

## Staining

### -Primary

Add the full amount of antibody to 50  $\mu$ l of permeabilization buffer and add this to the 50  $\mu$ 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  $\mu$ 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.