## 1. Prepare cells

> More detail you can view Sample Preparation for Flow Cytometry.

1) Collect cells, filtere through a 200-mesh sieve and collect the filtrate.Centrifuge at 300 g for 5 min, and discard the supernatant.

2) Add cell staining buffer(or PBS with 0.1% BSA) to resuspend the sample.

## 2. Cell Counting

After counting the suspension with a hemocytometer or other instruments, adjust the cell concentration to about 1 × 107/mL.

## 3. Set Sample and Control

| Groups | Tubes |
|---|---|
| Controls | Blank |
| | Single staining control |
| | Isotype control |
| | FMO |
| | Biological control |
| Sample | Experimental sample |

## 4. Block Fc Receptor

Block Fc receptors may reduce nonspecific immunofluorescent staining.

For Mouse cells: purified Anti-Mouse CD16/CD32 antibody specific for FcγR III/II can be used to block nonspecific staining of antibodies. Thus, block Fc receptors by pre-incubating cells with 0.5-1μg Anti-Mouse CD16/CD32 in 100 μL volume for 10 min at room temperature.

For Human and Rat cells: Pre-incubate the cells with excess irrelevant purified Ig from the same species and same isotype as the antibodies used for immunofluorescent staining or serums from the same species as the antibody used.

## 5. Cell Surface Staining

1). Add the antibody according to the recommended dosage of the instructions and mix well.

2). Incubate at 4°C for 30 min in the dark.

## 6. Detection

1). Add cell staining buffer, centrifuge at 300 g for 5 min, and discard the supernatant.

2). Add 200 μL cell staining buffer to resuspend the sample.

3). Adjust instrument parameters,detection.