1. Prepare cells

(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 [E-CK-A107] (or PBS with 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[E-AB-F0997A] 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 5 μL corresponding antibody to each sample tube except blank.

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

6. Fixation and Permeabilization

If the markers are those in the intracellular (e.g.IFN-γ,IL-4,IL-17), please refer to the Cells Intracellular Targets Staining for Flow Cytometry.

(1). Dilute Fixation and Permeabilization Solution[E-CK-A108] according to the manual.

(2). Add 1 mL cell staining buffer [E-CK-A107] (or PBS with 1% BSA) to each tube, centrifuge at 300×g for 5 min, and discard the supernatant.

(3). Add 100 µL cell staining buffer [E-CK-A107] (or PBS with 1% BSA) to resuspend the sample.

(4). Add 1 mL 1× Fixation Working Solution to each tube, mix gently.

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

(6). Centrifuge at 600×g for 5 min, and discard the supernatant.

(7). Add 2 mL 1× Permeabilization Working Solution to each tube, mix gently.

(8). Centrifuge at 600×g for 5 min, and discard the supernatant.

(9). Repeat (7)(8) one more time.

7. Cell Intracellular Staining

(1). Add 100 µL 1× Permeabilization Working Solution to each tube, resuspend the sample.

(2). Add 5 µL corresponding antibody to the tube required.

(3). Incubate at RT for 30 min in the dark.

(4). Add 1× Permeabilization Working Solution to each tube, resuspend the sample.

(5). Centrifuge at 600×g for 5 min, and discard the supernatant.

8. Detection

(1). Add 200 µL cell staining buffer [E-CK-A107] (or PBS with 1% BSA) to resuspend the sample.

(2). Adjust instrument parameters, detection.