Protocol for FCM

Experimental instruments

-80℃ refrigerator; centrifuge; clean bench; CO2 cell incubator; flow cytometer: NovoCyte 2060R

Cell preparation

Resuspension cells with PBS and count them, taking 5×10^5~1×10^6 cells per 100ul, add into 1.5mL EP tube or 2mL EP tube.

Staining

1. Primary antibody incubation: The blank control tube don't add antibody. The isotype control tube add isotype control antibody. The test tube add primary antibody. Mix well, and incubate at 4℃ for 30 minutes. Shake the tube every 10 minutes during incubation to reduce cell precipitation and allow it to fully react with the antibodies.
2. Cell cleaning: Add 1mL 2% BSA (prepared with PBS) to each tube, centrifuge at 1000rpm for 5 minutes, and discard the supernatant.
3. Cell resuspension: Resuspended cells using 100 μL 2% BSA (prepared with PBS).
4. Secondary antibody incubation: The isotype control and test tubes add fluorescent labeled secondary antibody. Mix well, and incubate at 4℃ in dark for 30 minutes. Shake the tubes every 10 minutes during incubation.
5. Cell cleaning: Add 1mL 2% BSA (prepared with PBS) to each tube,

centrifuge at 1000rpm for 5 minutes, discard the supernatant, and repeat cleaning once.

6. Cell resuspension: Resuspended cells using 100μL 2% BSA (prepared with PBS).

Detection

Take 50-100μL (about 10^4~10^5 cells) cell resuspension. The blank tube and the isotype control tube are tested first, and then test the sample tube.

Notes:

1. Pay attention to avoiding light during the use of fluorescent secondary antibodies.

2. If the antibody is directly coupled, remove the secondary antibody step and pay attention to avoiding light.