Intracellular Fixation/Permeabilization Buffer Kit

Catalog No.: K082

Size: 50T/100T/500T

Kit components:

Reagents	50T	100T	500T	Storage
Fixation buffer	10mL	20mL	50mL*2	2-8°C
Permeabilization Buffer (5×)	15mL	30mL	50mL*3	2-8°C

Storage:

2-8°C for 12 months

Introduction:

Intracellular Fixation/Permeabilization Buffer Kit has been formulated and optimized for staining intracellular antigens such as cytokines and chemokines.

Instructions:

Dilute Permeabilization Buffer (5×) with deionized water to 1×Permeabilization Working Solution before use.

Note: It is recommended that 1×permeabilization working solution should be prepared before use and used up within 3 days as far as possible.

Assay Protocol:

1. Take 1×10^6 cells in 100 μL suspension into the tube per sample.
2. [Optional] Stain cells with a Fixable Viability Dye (self-prepared).
3. [Optional] Block Fc receptors in cell suspensions according to experimental requirements.
4. Stain cell surface markers as need.
5. After incubating with the cell surface marker, add 2 mL of PBS (with 1% BSA, self-prepared) or Cell Staining Buffer [K079], centrifuge at 300×g for 5 min, discard the supernatant.

6. Resuspend the cells with 200 μL of PBS (with 1% BSA, self-prepared) or Cell Staining Buffer [K079]. Then add 200μL of Fixation Buffer, incubate the cells at room temperature for 30~60 min in the dark (please extend the incubation time to 60 min when the room temperature is lower than 25°C).

7. Add 1 mL of 1×Permeabilization Working Solution to each tube and mix fully, centrifuge at 600×g for 5 min and discard the supernatant.

8. Resuspend the cells with 100 μL of 1×Permeabilization Working Solution. Add the appropriate volume of intracellular antibody or corresponding isotype control and incubate at least 30 min at room temperature in the dark.

9. Add 2 mL of PBS (with 1% BSA) or Cell Staining Buffer [K079] to each tube and centrifuge at 600×g for 5 min, discard the supernatant.

10. Resuspend the cells with appropriate PBS (with 1% BSA) or Cell Staining Buffer [K079], then analyze the samples by flow cytometry.

Note:

1.It is normal for the Permeabilization Buffer (5×) to have precipitation, and it will not affect the use effect.

2.For samples with red blood cells, please lyse red blood cells first.

3.The fixation and permeabilization steps may alter the light scatter properties of cells and may increase non-specific background staining. The addition of BSA or fetal calf serum (FBS) in the staining buffer help to reduce non-specific background. It is recommended to use Fixable Viability Dye to eliminate the interference of dead cells in the data analysis process.

4.This product is compatible with most commercially available flow antibodies, but some antigenic determinants are sensitive to fixatives and the fixation time needs to be optimized for the situation.

5.For your safety and health, please wear the lab coat and disposable gloves before the experiments.