Intracellular Fixation/Permeabilization Buffer Kit (Ready To Use)

Catalog No.: K082R

Size: 50T/100T/500T

Kit components:

Reagents	50T	100T	500T	Storage
Fixation Buffer	10 mL	10 mL*2	50 mL*2	2-8°C, shading light
Permeabilization Buffer	5 mL	5 mL*2	50 mL	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.

Assay Protocol:

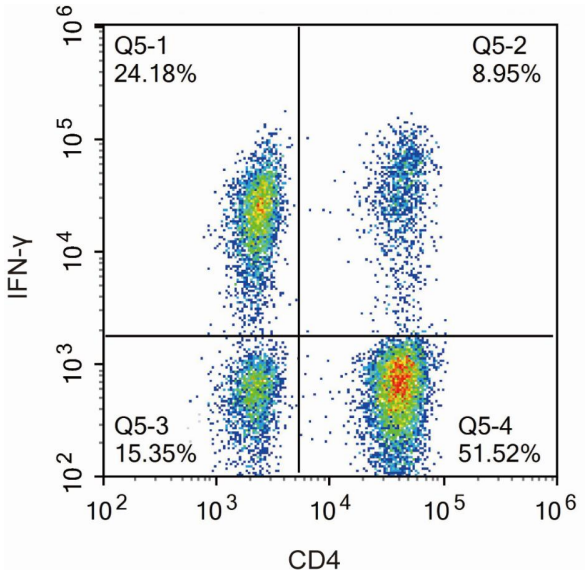
1. Take 1×10^6 cells in 100 µL suspension into the tube per sample.
2. [Optional] Stain cells with a Fixable Viability Dye (self-prepared).
3. [Optional] Block Fc receptors in cell suspensions according to experimental requirements.
4. Stain cell surface markers as need.
5. After incubating with the cell surface marker, add 1 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). Centrifuge at 600×g for 5 min and discard the supernatant.
7. Add 1 mL of PBS (with 1% BSA) to each tube and mix fully, centrifuge at 600×g for 5 min and discard the supernatant.

Note: If it is too late to complete all steps, after adding PBS (with 1% BSA), the cells can be stored at 4 °C, and then centrifuged on the second day.

8. Resuspend the cells with 100 μL of Permeabilization Buffer. 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 1 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.



Human peripheral blood lymphocytes are stained with FineTest®488Anti-Human CD4 Antibody and PE Anti-Human IFN-γ Antibody followed by fixation and permeabilization using Intracellular Fixation/Permeabilization Buffer Kit.

Note:

1. This product is for scientific research only.

2. 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 amino reactive dead cell identification dye to eliminate the interference of dead cells in the data analysis process.

3. This product is compatible with most commercially available flow antibodies, but some antigenic determinants are sensitive to fixatives and and are not compatible.

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

5. Fixation buffer and Permeabilization Buffer are both ready-to-use.