Intracellular Fixation/Permeabilization Buffer Kit

Cat. No: E-CK-A109 **Size: 50 Assays/100 Assays/500 Assays**

Cat.	Products	50 Assays	100 Assays	500 Assays	Storage
E-CK-A109A	Fixation Buffer	10 mL	20 mL	50 mL×2	2~8°C
E-CK-A109B	Permeabilization Buffer (5×)	15 mL	30 mL	50 mL×3	2~8°C
Manual			One Copy		

Storage

Store at 2~8°C for one year in the dark.

Introduction

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

Instructions

Dilute Permeabilization Buffer (5×) [E-CK-A109B] with deionized water to 1×Permeabilization Working Solution before use.

For example, take 1 mL Permeabilization Buffer (5×) [E-CK-A109B] to 4 mL deionized water and mix fully to prepare 1× Permeabilization Working Solution.

Note: It is recommended that 1×Permeabilization Working Solution be used up within 3 days.

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Experimental Procedure

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 [E-CK-A107], 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 [E-CK-A107]. 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 [E-CK-A107] 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 [E-CK-A107], then analyze the samples by flow cytometry.

Cautions

1. This kit is for research use only.
2. Permeabilization Buffer (5×) may precipitate, and it will not affect the use effect.
3. For samples with red blood cells, please lyse red blood cells first.
4. 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.
5. 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.
6. For your safety and health, please wear the lab coat and disposable gloves before the experiments.
7. For conventional fixation of cells after cell surface antibody staining, the fixation conditions should follow the instructions in Section 6 of the aforementioned operating guidelines. Under special circumstances, such as when cell surface markers are fixation-resistant and delayed detection is required, the ratio of cell suspension to fixative may be adjusted to 3:1, after thorough mixing, fixation should be performed overnight at 4℃, followed by subsequent procedures.

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