Foxp3/Transcription Factor Staining Kit

Cat. No: E-CK-A108 **Size: 20 Assays**

Cat.	Products	20 Assays	Storage
E-CK-A108A	Fixation Concentrate (4×)	5 mL	2~8 ℃
E-CK-A108B	Fixation Dilution Solution	15 mL	2~8 ℃
E-CK-A108C	Permeabilization Buffer (10×)	17 mL	2~8 ℃
	Manual	One Copy	

Storage

Store at 2~8 ℃ for six months in the dark. Avoid freeze / thaw cycles.

Introduction

Elabscience® Foxp3 / Transcription Factor Staining Kit has been formulated and optimized for staining with antibodies to transcription factors and nuclear proteins, such as Foxp3 and STAT3.

Instructions

Dilute Fixation Concentrate (4×) with Fixation Dilution Solution to 1× Fixation Working Solution before use.

For example, take 1 mL Fixation Concentrate (4×) [E-CK-A108A] and add it to 3 mL Fixation Dilution Solution [E-CK-A108B] to get 4 mL 1× Fixation Working Solution. Each sample requires 1 mL of 1× Fixation Working Solution.

Dilute Permeabilization Buffer (10×) with ddH_2O to 1×Permeabilization Working Solution before use.

For example, take 1 mL Permeabilization Buffer (10×) [E-CK-A108C], and add it to 9 mL ddH_2O to get 10 mL 1× Permeabilization Working Solution. Each sample requires 6.5 mL of 1× Permeabilization Working Solution.

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Experimental Procedure

1. Add the single-cell suspension into tubes, 1×10^6 cells in 100 µL suspension per tube.
2. [Optional] Stain cells with a Fixable Viability Dye.
3. [Optional] Block Fc receptors in cell suspensions according to experimental requirements.
4. Stain cell surface markers. Refer to the FCM protocol (Staining Cell Surface Targets for Flow Cytometry).
5. After incubating with the cell surface marker, add 1 mL of Cell Staining Buffer [E-CK-A107], centrifuge samples at 300×g for 5 min, discard the supernatant, then resuspend the cells with 100 µL of Cell Staining Buffer [E-CK-A107].
6. Add 1 mL of 1×Fixation Working Solution to each tube and mix fully, incubate the cells at 4℃ for 30 min, then centrifuge at 600×g for 5 min and discard the supernatant.
7. Add 2 mL of 1×Permeabilization Working Solution to each tube and mix fully, centrifuge at 600×g for 5 min and discard the supernatant.
8. Repeat Step 7.
9. Resuspend the cells with 100 µL of 1×Permeabilization Working Solution.
10. Without washing, add the recommended amount of directly FCM antibody for detection of intracellular antigen(s) to cells and incubate for at least 30 min at room temperature in the dark.
11. Add 2 mL of 1×Permeabilization Working Solution to each tube and centrifuge at 600×g for 5 min at room temperature. Discard the supernatant.
12. Resuspend the cells with appropriate Cell Staining Buffer [E-CK-A107], then analyze the samples by flow cytometer.

Cautions

1. It is normal for the Permeabilization Buffer (10×) to have precipitation, and it will not affect the use effect.
2. For maximal assay performance, this reagent should be used within 6 months. Avoid freeze / thaw cycles.
3. The fixation and permeabilization steps that are required for the detection of intracellular antigens may alter the light scatter properties of cells and may increase non-specific background staining. Including extra proteins such as BSA or fetal calf serum (FCS) in the staining buffer may help reduce non-specific background. The use of Fixable Viability Dyes is recommended to help eliminate dead cells during the analysis.
4. For your safety and health, please wear the lab coat and disposable gloves before the experiments.

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