Foxp3/Transcription Factor Staining Kit

Catalog No.: K081

Size: 20T

Kit components:

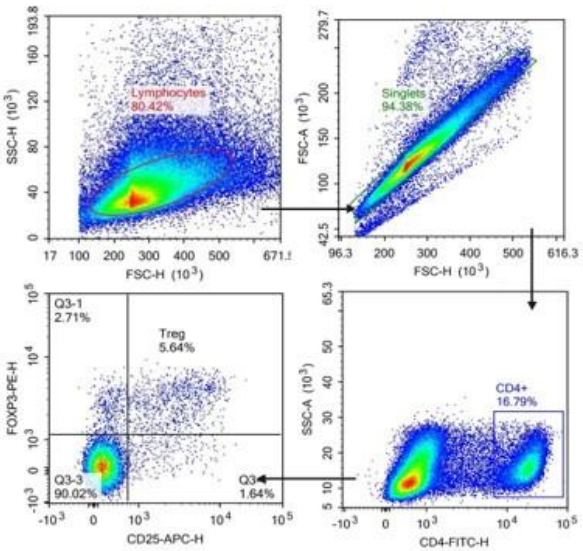
Reagents	20T	Storage
Fixation Concentrate (4×)	5mL	2-8°C
Fixation Dilution Solution	15mL	2-8°C
Permeabilization Buffer (10×)	17mL	2-8°C

Storage:

2-8°C for 6 months

Introduction:

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.



To detect Treg cells in mouse spleen, the CD4+ T cells that are double-positive for CD25 and Foxp3 are considered Treg cells.

Instructions:

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

Dilute Permeabilization Buffer (10×) with distilled water to 1× Permeabilization Working Buffer before use.

Assay Protocol:

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 [K079], centrifuge samples at 300×g for 5 min, discard the supernatant, then resuspend the cells with 100 µL of Cell Staining Buffer [K079].

6. Add 1 mL of 1×Fixation Working Solution to each tube and mix fully, incubate the cells at 4°C for 30 min, then centrifuge at 600×g for 5 min and discard the supernatant.

7. Add 2 mL of 1×Permeabilization Working Buffer 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 Buffer.

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 Buffer 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 [K079], then analyze the samples by flow cytometer.

Note:

1.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 Dye is recommended to help eliminate dead cells during the analysis.

2.This kit is for research use only. Not for use in diagnosis and therapy.

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