True-Nuclear™ Transcription Factor Staining Protocol for 5 mL Tubes Version 2

BioLegend, Inc.

Abstract

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Guidelines

NOTE: For flow cytometric staining with this clone, True-Nuclear™ Transcription Buffer Set (Cat. No. 424401) offers improved staining and is highly recommended over the Foxp3 Fix/Perm Buffer Set (Cat. No. 421403) and the Nuclear Factor Fixation and Permeabilization Buffer Set (Cat. No. 422601).

Reagent List:
• True-Nuclear™ Transcription Factor Buffer Set (Cat. No. 424401)
• Cell Staining Buffer (Cat. No. 420201)

Materials

True-Nuclear™ Transcription Factor Buffer Set 424401 by BioLegend

Cell Staining Buffer 420201 by BioLegend

Protocol

Step 1.

Perform cell surface staining as described in BioLegend's Cell Surface Immunofluorescence Staining Protocol.

Step 2.

Add 1 mL of the Transcription Factor 1X Fix solution to each tube, vortex and incubate at roomtemperature in the dark for 45-60 minutes.

DURATION
00:04:00

Step 3.

Without washing, add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.

Step 4.
Centrifuge tubes at 300-400 x g at room temperature for 5 minutes, and discard the supernatant.

DURATION
00:05:00

Step 5.
Add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.

Step 6.
Centrifuge tubes at 300-400 x g at room temperature for 5 minutes, and discard the supernatant.

DURATION
00:05:00

Step 7.
Resuspend the cell pellet in 100 µL of the Transcription Factor 1X Perm Buffer.

Step 8.
Add the appropriate amount of fluorochrome conjugated antibody for detection of intracellularantigen(s) to each tube and incubate in the dark at room temperature for at least 30 minutes.

DURATION
00:30:00

Step 9.
Add 2 mL of the Transcription Factor 1X Perm Buffer to each tube.

Step 10.
Centrifuge tubes at 300-400 x g at room temperature for 5 minutes, and discard thesupernatant.

DURATION
00:05:00

Step 11.
Add 2 mL of cell staining buffer (Cat. No. 420201).

REAGENTS
Cell Staining Buffer 420201 by BioLegend

Step 12.
Centrifuge tubes at 300-400 x g at room temperature for 5 minutes, and discard thesupernatant.

DURATION
00:05:00

Step 13.
Resuspend in 0.5 mL cell staining buffer then acquire the tubes on a flow cytometer.