True-Nuclear™ Transcription Factor Staining Protocol for 96-Well, U-Bottom Plate 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)

Before start

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).

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.
After the last wash, discard the supernatant, and gently vortex the samples to dissociate the cell pellet.

Step 3.
Add 200 µL of the Transcription Factor 1X Fix solution to each well. Gently pipette to ensure cells are fully resuspended.

Step 4.

Incubate at room temperature in the dark for 45-60 minutes.

DURATION
00:45:00

Step 5.
Centrifuge the plate at 300-400 x g at room temperature for 5 minutes, discard the supernatant, and gently vortex to dissociate the cell pellet.

DURATION
00:05:00

Step 6.
Add 200 µL of the Transcription Factor 1X Perm Buffer to each well. (wash 1/3)

Step 7.
Centrifuge the plate at 300-400 x g at room temperature for 5 minutes, discard the supernatant, and gently vortex to dissociate the cell pellet. (wash 1/3)

DURATION
00:05:00

Step 8.
Add 200 µL of the Transcription Factor 1X Perm Buffer to each well. (wash 2/3)

Step 9.
Centrifuge the plate at 300-400 x g at room temperature for 5 minutes, discard the supernatant, and gently vortex to dissociate the cell pellet. (wash 2/3)

DURATION
00:05:00

Step 10.
Add 200 µL of the Transcription Factor 1X Perm Buffer to each well. (wash 3/3)

Step 11.
Centrifuge the plate at 300-400 x g at room temperature for 5 minutes, discard the supernatant, and gently vortex to dissociate the cell pellet. (wash 3/3)

DURATION
00:05:00

Step 12.
Add the appropriate amount of fluorochrome conjugated antibody for detection of intracellular antigen(s) to each well and incubate in the dark at room temperature for at least 30 minutes.

DURATION
00:30:00

Step 13.
Add 200 µL of the Transcription Factor 1X Perm Buffer to each well. (wash 1/3)

Step 14.

Centrifuge the plate at 300-400 x g at room temperature for 5 minutes, discard thesupernatant, and gently vortex to dissociate the cell pellet. (wash 1/3)

DURATION
00:05:00

Step 15.
Add 200 µL of the Transcription Factor 1X Perm Buffer to each well. (wash 2/3)

Step 16.
Centrifuge the plate at 300-400 x g at room temperature for 5 minutes, discard thesupernatant, and gently vortex to dissociate the cell pellet. (wash 2/3)

DURATION
00:05:00

Step 17.
Add 200 µL of the Transcription Factor 1X Perm Buffer to each well. (wash 3/3)

Step 18.
Centrifuge the plate at 300-400 x g at room temperature for 5 minutes, discard thesupernatant, and gently vortex to dissociate the cell pellet. (wash 3/3)

DURATION
00:05:00

Step 19.
Resuspend in cells in appropriate volume of cell staining buffer and acquire samples on a flowcytometer.

REAGENTS
Cell Staining Buffer 420201 by BioLegend