

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

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 30-60 minutes.

 **DURATION**

00:30:00

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 intracellular antigen(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 the supernatant.

 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 the supernatant.

 DURATION

00:05:00

Step 13.

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