True-Nuclear™ Transcription Factor Staining Protocol for 5 mL Tubes V.4

DOI
dx.doi.org/10.17504/protocols.io.bacsiawe

Sam Li[1]

[1]BioLegend

BioLegend
Tech. support email: **tech@biolegend.com**

Sam Li
BioLegend

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External link: https://www.biolegend.com/protocols/true-nuclear-transcription-factor-staining-protocol-for-5ml-tubes/4267/

Protocol Citation: Sam Li . True-Nuclear™ Transcription Factor Staining Protocol for 5 mL Tubes. **protocols.io https://dx.doi.org/10.17504/protocols.io.bacsiawe**

Created: December 10, 2019

Last Modified: December 10, 2019

Protocol Integer ID: 30834

Keywords: true nuclear, transcription factor, flow cytometry staining

Guidelines

General Tips and FAQ:

What are some improvements in the True-Nuclear™ Buffer compared to the Foxp3 Fix/Perm (Cat. No. 421403) and the Nuclear Factor Fixation and Permeabilization Buffer Sets (Cat. No.422601)?

- Less background for nuclear staining (e.g.- Foxp3) staining, offering improved signal/noise ratio for better population distinction
- Minimal effects on surface staining, especially those stained using tandem fluorophores such as APC/FIRE™ 750

I am observing high background staining. What can I do to reduce it?

- Longer fixation time – as noted in the fixation step, a longer fixation period can help ameliorate high background

Is theTrue-Nuclear™ Buffer set recommended for intracellular cytokine staining?

- No: The permeabilization conditions required for staining transcription factors using True-Nuclear™ (or Foxp3 Fix/Perm and Nuclear Factor Fixation and Permeabilization Buffer Sets) is much harsher than those recommended for staining cytokines (i.e.- cells become more porous). This can lead to cytokine leakage upon permeabilization, resulting in loss of signal. For staining intracellular cytokines, we recommend using the **Fixation (Cat. No. 420801)** and **Permeabilization Wash Buffers (Cat. No. 421002)** and following our intracellular staining protocol, which can be found **here**.

Any known nuclear targets not suited for detection using the True-Nuclear™ buffer?

- Ki-67: You can find our recommended protocol for Ki-67 staining **here.**

Materials

MATERIALS

Cell Staining Buffer **BioLegend Catalog #**420201

True-Nuclear™ Transcription Factor Buffer Set **BioLegend Catalog #**424401

STEP MATERIALS

Cell Staining Buffer **BioLegend Catalog #** 420201

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

2 Add 1mL of the True-Nuclear™ 1X Fix Concentrate to each tube, vortex and incubate at room temperature in the dark for 45-60 minutes. If necessary, the protocol can be suspended at this point. After discarding supernatant, re-suspend cells in CytoLast™ Buffer (Cat. No. **422501**) or equivalent. Samples can be stored at 4°C for 12-18 hours, protected from light and plastic-wrapped to protect buffer evaporation.

3 Without washing, add 2mL of the True-Nuclear™ 1X Perm Bufferto each tube.

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

5 Add 2mL of the True-Nuclear™ 1X Perm Buffer to each tube.

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

7 Resuspend the cell pellet in 100µL of the True-Nuclear™ 1X Perm Buffer.

8 Add the appropriate amount of fluorochrome conjugated antibody diluted in True-Nuclear™ 1X Perm Buffer for detection of intracellular antigen(s) to each well and incubate in the dark at room temperature for at least 30 minutes.

9 Add 2mL of the True-Nuclear™ 1X Perm Buffer to each tube.

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

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

12 Centrifuge tubes at 300-400xg at room temperature for 5 minutes, and discard the supernatant.

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