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

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1 Works for me
[dx.doi.org/10.17504/protocols.io.bacsiawe](https://doi.org/10.17504/protocols.io.bacsiawe)

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EXTERNAL LINK

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

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 the True-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

NAME	CATALOG #	VENDOR
Cell Staining Buffer	420201	BioLegend
True-Nuclear™ Transcription Factor Buffer Set	424401	BioLegend

- 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 Buffer to 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.



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