🔒 True-Nuclear™ Transcription Factor Staining Protocol for 96-Well, U-Bottom Plate V.5 👤

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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](#).

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MATERIALS

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

Cell Staining Buffer BioLegend Catalog #420201

STEP MATERIALS

Cell Staining Buffer BioLegend Catalog #420201

- 1 Perform cell surface staining as described in BioLegend's Cell Surface Flow Cytometry Protocol.
- 2 After the last wash, discard the supernatant, and gently vortex the samples to dissociate the cell pellet.
- 3 Add 200µL of the True-Nuclear™ 1X Fix Concentrate to each well. Gently pipette to ensure cells are fully resuspended. Incubate at room temperature in the dark for 45-60 minutes.
- 4 Centrifuge the plate at 300-400xg at room temperature for 5 minutes, discard the supernatant, and gently vortex to dissociate the cell pellet. Tip: 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.
- 5 Add 200µL of the True-Nuclear™ 1X Perm Buffer to each well.
- 6 Centrifuge the plate at 300-400xg at room temperature for 5 minutes, discard the supernatant, and gently vortex to dissociate the cell pellet.
- 7 Repeat steps 5-6 for 2 additional times, for a total of 3 washes using 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 200µL of the True-Nuclear™ 1X Perm Buffer to each well.
- 10** Centrifuge the plate at 300-400xg at room temperature for 5 minutes, discard the supernatant, and gently vortex to dissociate the cell pellet.
- 11** Repeat steps 9-10 for 2 additional times, for a total of 3 washes using the True-Nuclear™ 1X Perm Buffer.
- 12** Resuspend in cells in appropriate volume of cell staining buffer and acquire samples on a flow cytometer.