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

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Version 4

Working

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EXTERNAL LINK

https://www.biolegend.com/protocols/true-nuclear-transcription-factor-staining-protocol-for-96-well-u-bottom-plate/4246/

PROTOCOL STATUS

**Working**

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 # | |
|---|---|---|
| True-Nuclear™ Transcription Factor Buffer Set | 424401 | by BioLegend |
| Cell Staining Buffer | 420201 | by BioLegend |

1 Perform cell surface staining as described in BioLegend's Cell Surface Immunofluorescence Staining 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. 01:00:00

**Tip:** Longer fixation time [between 50-60 minutes] is recommended to reduce background staining.

4 Centrifuge the plate at 300-400xg at room temperature for 5 minutes, discard the supernatant, and gently vortex to dissociate the cell pellet. 00:05:00

**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.
00:05:00

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.
00:30:00

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.
00:05:00

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.