Intracellular Staining With True-Phos™ Perm Buffer in Cell Suspensions V.2

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MATERIALS

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Cell Staining Buffer **BioLegend Catalog #420201**

Fixation Buffer **BioLegend Catalog #420801**

DOI:

[dx.doi.org/10.17504/protocol.s.io.bac4iayw](https://doi.org/10.17504/protocol.s.io.bac4iayw)

External link:

<https://www.biolegend.com/protocols/intracellular-staining-with-true-phos-perm-buffer-in-cell-suspensions-protocol/4262/>

Protocol Citation: Sam Li . Intracellular Staining With True-Phos™ Perm Buffer in Cell Suspensions.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.bac4iayw>

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Created: Dec 10, 2019

Last Modified: Dec 10, 2019

PROTOCOL integer ID:

30844

Keywords: true-phos, perm buffer, intracellular flow cytometry staining

Buffer Preparation:

- 1 Warm Fixation Buffer (BioLegend Cat [420801](#)). For each 1×10^6 cells aliquot 0.5mL of buffer and warm to 37°C.

- 2 Chill True-Phos™ Perm Buffer to -20°C. For each 1×10^6 cells aliquot 1.0ml of True-Phos™ Perm Buffer and chill to -20°C.

Sample Preparation:

- 3 Prepare a single cell suspension with the sample of interest (Human PBMC, splenocytes, cell lines, etc).

Tips:
 - Prepare two aliquots, Negative control: untreated, Positive control: treated with stimuli.
 - Incubate the cells with the appropriate stimuli, at the suitable temperature and time.
- 4 Fix the cells immediately after treatment by adding an equal volume of pre-warmed Fixation Buffer. Gently pipette to ensure thorough mixing.
- 5 Incubate at 37°C for 15 minutes to ensure cells are properly fixed.
- 6 Centrifuge cells at 350xg at room temperature for 5 minutes, decant supernatant, vortex to resuspend cell pellet.

Staining with Specific Antibodies:

- 7 Add sufficient Cell Staining Buffer to wash the cells (approximately 2ml for each 1×10^6 cells, BioLegend Cell Staining Buffer recommended, Cat [420201](#)), centrifuge at 350xg at room temperature for 5 minutes and decant supernatant. Repeat, for a total of two washes.
- 8 Gently pipette cells using residual volume to resuspend cell pellet. Note: if cells are not fully resuspended, True-Phos™ Perm Buffer addition will cause significant cell loss.
- 9 While vortexing, permeabilize cells by adding pre-chilled True-Phos™ Perm Buffer. Example: 10×10^6 cells should be permeabilized with 10mL of pre-chilled True-Phos™ Perm Buffer.

- 10** Incubate at -20°C for at least 60 minutes to ensure cells are properly permeabilized. Note: cells can be stored in the True-Phos™ Perm Buffer overnight at -20°C.
- 11** Centrifuge cells at 1000xg at room temperature for 5 minutes, decant supernatant, vortex to resuspend cell pellet.
- 12** Add sufficient Cell Staining Buffer to wash the cells, centrifuge cells at 1000xg at room temperature for 5 minutes, decant supernatant. Repeat, for a total of two washes.
- 13** Resuspend the cells in Cell Staining Buffer at a concentration of 10×10^6 cells/ml.
- 14** Transfer 100µL (or 1×10^6 cells) to a 12 x 75mm tube.
- 15** Add antibody cocktail(s) to appropriate tubes, vortex to mix, and incubate for 30 minutes at room temperature in the dark.
- 16** Add 2mL of Cell Staining Buffer, centrifuge cells at 1000xg at room temperature for 5 minutes, decant supernatant. Repeat, for a total of two washes.
- 17** Resuspend cells in approximately 500µl of Cell Staining Buffer and analyze with a flow cytometer.