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

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1 Works for me dx.doi.org/10.17504/protocols.io.bac4iayw

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EXTERNAL LINK

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

MATERIALS

NAME	CATALOG #	VENDOR
Cell Staining Buffer	420201	BioLegend
Fixation Buffer	420801	BioLegend

Buffer Preparation:

1 Warm Fixation Buffer (BioLegend Cat 420801). For each 1 x 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 x 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 x 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 x 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 x 10^6 cells/ml.

14 Transfer 100µL (or 1 x 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.