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

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Abstract

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Protocol

Buffer preparation

Step 1.

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

Buffer preparation

Step 2.

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

Sample Preparation

Step 3.

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

Tips:

1. Prepare two aliquots, Negative control: untreated, Positive control: treated with stimuli.
2. Incubate the cells with the appropriate stimuli, at the suitable temperature and time.

Sample Preparation

Step 4.

Fix the cells immediately after treatment by adding an equal volume of pre-warmed Fixation Buffer. Gently pipette to ensure thorough mixing.

Sample Preparation

Step 5.

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Incubate at 37°C for 15 minutes to ensure cells are properly fixed.

DURATION
00:15:00

Sample Preparation

Step 6.

Centrifuge cells at 350 x g at room temperature for 5 minutes, decant supernatant, vortex to resuspend cell pellet.

DURATION
00:05:00

Staining with Specific Antibodies

Step 7.

Add sufficient Cell Staining Buffer to wash the cells (approximately 2 ml for each 1 x 10^6 cells, BioLegend Cell Staining Buffer recommended, Cat#420201).

Centrifuge at 350 x g at room temperature for 5 minutes and decant supernatant. Repeat, for a total of two washes.

Staining with Specific Antibodies

Step 8.

Gently pipette cells using residual volume to resuspend cell pellet.

NOTES
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Note: if cells are not fully resuspended, True-Phos™ Perm Buffer addition will cause significant cell loss

Staining with Specific Antibodies

Step 9.

While vortexing, permeabilize cells by adding pre-chilled True-Phos™ Perm Buffer.

Example:10 x 10^6 cells should be permeabilized with 10 mL of pre-chilled True-Phos™ Perm Buffer.

Staining with Specific Antibodies

Step 10.

Incubate at -20°C for 60 minutes to ensure cells are properly permeabilized.

DURATION
01:00:00

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Staining with Specific Antibodies

Step 11.

Centrifuge cells at 1000 x g at room temperature for 5 minutes, decant supernatant, vortex to resuspend cell pellet.

DURATION
00:05:00

Staining with Specific Antibodies

Step 12.

Add sufficient Cell Staining Buffer to wash the cells, centrifuge cells at 1000 x g at room temperature for 5 minutes, decant supernatant. Repeat, for a total of two washes.

Staining with Specific Antibodies

Step 13.

Resuspend the cells in Cell Staining Buffer at a concentration of 10 x 10^6 cells/ml.

Staining with Specific Antibodies

Step 14.

Transfer 100 uL (or 1 x 10^6 cells) to a 12 x 75 mm tube.

Staining with Specific Antibodies

Step 15.

Add antibody cocktail(s) to appropriate tubes, vortex to mix, and incubate for 30 minutes at room temperature in the dark.

DURATION
00:30:00

Staining with Specific Antibodies

Step 16.

Add 2 mL of Cell Staining Buffer, centrifuge cells at 1000 x g at room temperature for 5 minutes, decant supernatant. Repeat, for a total of two washes.

Staining with Specific Antibodies

Step 17.

Resuspend cells in approximately 500 ml of Cell Staining Buffer and analyze with a flow cytometer.

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