Intracellular Staining With True-Phos™ Perm Buffer in Whole Blood

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Abstract

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Protocol

Buffer Preparation

Step 1.

Warm 1 X RBC Lysis/Fixation Solution (Cat# 422401, 10X solution). For each 0.1 mL of whole blood, aliquot 2 mL of 1 X RBC Lysis/Fixation Solution to a 50 mL conical tube and warm to 37°C.

Buffer Preparation

Step 2.

Chill True-Phos™ Perm Buffer to -20°C. For each 0.1 mL of whole blood, aliquot 1.0 mL of True-Phos™ Perm Buffer and chill to -20°C.

Sample Preparation

Step 3.

Aliquot 0.1 mL of whole blood (heparin) into a 50 mL conical tube for each test

Tips:

- 22 tests (or 2.2 mL of whole blood) are the maximum number of tests that can be processed in a 50 mL conical, due to volume constraints.

-Prepare two aliquots: Negative control: untreated, Positive control: treated with stimuli

-Incubate the cells with the appropriate stimuli, at the suitable temperature and time.

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Sample Preparation

Step 4.

Fix the cells immediately after treatment by pre-warmed 1 X RBC Lysis/Fixation Solution. Gently pipette to ensure thorough mixing

Sample Preparation

Step 5.

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: for 1 mL of whole blood, permeabilize with 10 mL of pre-chilled True-Phos™ Perm Buffer

Staining with Specific Antibodies

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Step 10.

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

DURATION
01:00:00

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 a volume of Cell Staining Buffer equivalent to the starting volume of blood

Example: if starting volume of whole blood was 1 mL, resuspend cell pellet in 1 mL of Cell Staining Buffer

Staining with Specific Antibodies

Step 14.

Transfer 100 ml 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

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Step 17.

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

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