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Intracellular Staining With True-Phos™ Perm Buffer in Whole Blood V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bac6iaze

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EXTERNAL LINK

https://www.biolegend.com/protocols/intracellular-staining-with-true-phos-perm-buffer-in-whole-blood/4261/

MATERIALS

NAME	CATALOG #	VENDOR
Cell Staining Buffer	420201	BioLegend
RBC Lysis/Fixation Solution (10X)	422401	BioLegend
True-Phos™ Perm Buffer	425401	BioLegend

Buffer Preparation:

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

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

Sample Preparation:

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

Tips:
- 22 tests (or 2.2mL of whole blood) are the maximum number of tests that can be processed in a 50mL 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.

4 Fix the cells immediately after treatment by pre-warmed 1 X RBC Lysis/Fixation Solution. 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: for 1mL of whole blood, permeabilize 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 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.

14 Transfer 100µL 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.