

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

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Materials

MATERIALS



Cell Staining Buffer **BioLegend Catalog #420201**



RBC Lysis/Fixation Solution (10X) **BioLegend Catalog #422401**



True-Phos™ Perm Buffer **BioLegend Catalog #425401**



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 1×10^6 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 100 μL to a 12 \times 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 500 μL of Cell Staining Buffer and analyze with a flow cytometer.