Cell Fixation and Permeabilization Protocol using 70% Ethanol Version 2

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Abstract

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Protocol

Step 1.

Prepare 70% Ethanol and chill to -20°C

Note: Do not freeze ethanol for long-term storage).

Step 2.

Prepare target cells of interest and wash 2X with PBS, centrifuging at 350xg for 5 minutes.

DURATION
00:05:00

Step 3.

Discard supernatant and loosen the cell pellet by vortexing.

Step 4.

Add 3 ml cold 70% ethanol drop by drop to the cell pellet while vortexing.

Step 5.

Continue vortexing for 30 seconds.

DURATION
00:00:30

Step 6.

Incubate at -20°C for 1 hour

DURATION

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01:00:00

Step 7.

Wash 2X with BioLegend Cell Staining Buffer (Cat.#420201) and resuspend cells at 0.5-1.0 x 10^7 cells/ml.

Step 8.

Use 100 µl cell suspension/staining tube.

NOTES

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Please note that certain markers or fluors may not survive ethanol fixation. Protein-based fluors, like PE and APC, tend to have more difficulty, while synthetic fluors, like Brilliant Violet™, tend to have a higher chance of surviving the process.

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