

Ethanol Fixation Protocol

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Abstract

Cell Fixation and Permeabilization Protocol Using 70% Ethanol

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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

01:00:00

Step 7.

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

Step 8.

Use 100 μ l cell suspension/staining tube.