Dec 10, 2019

Cell Fixation and Permeabilization Protocol using 70% Ethanol V.3

Sam Li[1]

[1]BioLegend

1 Works for me dx.doi.org/10.17504/protocols.io.bacziax6

BioLegend

Sam Li
BioLegend

EXTERNAL LINK

https://www.biolegend.com/protocols/cell-fixation-and-permeabilization-protocol-using-70-ethanol/4252/

MATERIALS

NAME	CATALOG #	VENDOR
Cell Staining Buffer	420201	BioLegend

1 Prepare 70% Ethanol and chill to -20°C.Tip: Do not freeze ethanol for long-term storage.

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

3 Discard supernatant and loosen the cell pellet by vortexing.

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

5 Continue vortexing for 30 seconds and then incubate at -20°C for 1 hour.

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

7 Use 100µl cell suspension/staining tube.
Note: 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.