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Cell Fixation and Permeabilization Protocol using 70% Ethanol V.3 [↗](#)Sam Li<sup>1</sup><sup>1</sup>BioLegend

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Works for me

[dx.doi.org/10.17504/protocols.io.bacziax6](https://doi.org/10.17504/protocols.io.bacziax6)

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## 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<sup>7</sup> 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.



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