



Dec 10, 2019

Ki-67 Staining Protocol V.2 [↗](#)Sam Li¹¹BioLegend

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Works for me

[dx.doi.org/10.17504/protocols.io.bac2iaye](https://doi.org/10.17504/protocols.io.bac2iaye)

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EXTERNAL LINK

<https://www.biolegend.com/protocols/ki-67-flow-cytometry-staining-protocol/4263/>

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 3X with BioLegend's Cell Staining Buffer (Cat. No. [420201](#)) and then resuspend the cells at the concentration of 0.5-10 x 10⁶/ml.
- 7 Mix 100µl cell suspension with proper fluorochrome-conjugated Ki-67 antibody and incubate at room temperature in the dark for 30 minutes.
- 8 Wash 2X with BioLegend's Cell Staining Buffer and then resuspend in 0.5ml cell staining buffer for fluorescence activated cell sorting (FACS), or flow cytometric analysis.
Tip: Based on customer testing, Ki-67 staining is not recommended with our True-Nuclear™ Transcription Factor Buffer Set.



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