**Ki-67 Staining Protocol V.2**

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**Works for me**

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**EXTERNAL LINK**

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**PROTOCOL CITATION**

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**KEYWORDS**

ki-67, flow cytometry, cell division, proliferation, replicating

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**MATERIALS TEXT**

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Cell Staining

Buffer BioLegend Catalog #420201

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 10^6/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.