Ki-67 Staining Protocol V.2

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

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Materials

MATERIALS

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 × 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.