# Ki-67 Staining Protocol

**Kelsey Miller**

## Abstract

**Citation:** Kelsey Miller Ki-67 Staining Protocol. **protocols.io**
dx.doi.org/10.17504/protocols.io.fabbian
**Published:** 08 Jul 2016

## Protocol

**Step 1.**
Prepare 70% ethanol and chill at -20°C.

**Step 2.**
Prepare target cells of interest and wash 2X with PBS by centrifuge at 350xg for 5 minutes.

DURATION
00:05:00

**Step 3.**
Discard supernatant and loosen the cell pellet by vortexing.

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

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

DURATION
01:00:00

**Step 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.

**Step 7.**
Mix 100 µl cell suspension with proper fluorochrome-conjugated Ki-67 antibody and incubate at room temperature in the dark for 30 minutes.

DURATION
00:30:00

**Step 8.**
Wash 2X with BioLegend's Cell Staining Buffer (Cat. No. 420201) and then resuspend in 0.5 ml cell staining buffer for flow cytometric analysis.