SONY

Product Data Sheet

Alexa Fluor® 488 anti-human Ki-67

Catalog # / Size: 2352540 / 100 tests
2352535 / 25 tests

2352660 / 100 µg

Clone: Ki-67

Isotype: Mouse IgG1, κ

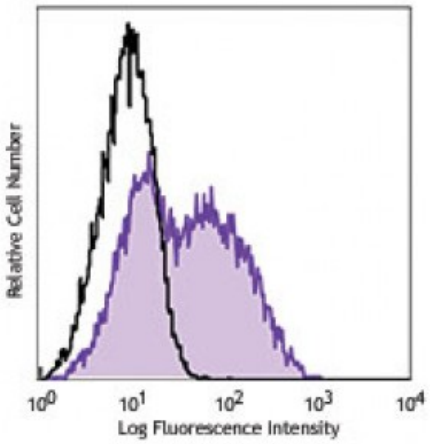
Immunogen: Nuclei of the Hodgkin lymphoma cell line L428

Reactivity: Human

Preparation: The antibody was purified by affinity chromatography, and conjugated with Alexa Fluor® 488 under optimal conditions.

Formulation: microg size: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
test sizes: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA (origin USA).

Concentration: microg sizes: 0.5 mg/ml
test sizes: lot-specific



PHA-activated human peripheral blood lymphocytes (3 days) were fixed and permeabilized with 70% ethanol, and then were stained with Ki-67 Alexa Fluor® 488 (filled histogram) or mouse IgG1, κ Alexa Fluor® 488 isotype control (open histogram)

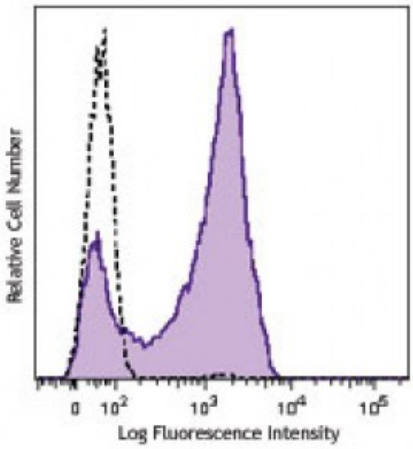
Applications:

Applications: Flow Cytometry

Recommended Usage: Each lot of this antibody is quality control tested by our Ki-67 staining protocol below. For flow cytometric staining using the microg size, the suggested use of this reagent is ≤1.0 microg per million cells in 100 microL volume. For flow cytometric staining using test sizes, the suggested use of this reagent is 5 microL per million cells or 5 microL per 100 microL of whole blood. For immunofluorescence microscopy, a concentration range of 1-5 µg/ml is recommended. It is recommended that the reagent be titrated for optimal performance for each application.

* Alexa Fluor® 488 has a maximum emission of 519 nm when it is excited at 488 nm.

Application Notes: Additional reported applications (for the relevant formats) include: immunohistochemical staining of frozen tissue sections1, Western blotting3, and immunofluorescence microscopy4.



3-day PHA-stimulated human peripheral blood lymphocytes were fixed and permeabilized with BioLegend FOXP3 buffer set, then stained with Ki-67 Alexa Fluor® 488 (filled histogram) or mouse IgG1, κ Alexa Fluor® 488 isotype control (open histogr

Ki-67 Staining Protocol:

1. Prepare 70% ethanol and chill at -20°C.
2. Prepare target cells of interest and wash 2X with PBS by centrifuge at 350x*g* for 5 minutes.
3. Discard supernatant and loosen the cell pellet by vortexing.
4. Add 3 ml 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 Cell Staining Buffer and then resuspend the cells at the concentration of 0.5-10 x 10^6/ml.
7. Mix 100 microL 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 Cell Staining Buffer and then resuspend in 0.5 ml cell staining buffer for flow cytometric analysis.

Application References:

1. Gerdes J, *et al.* 1983. *Int. J. Cancer* 31:13. (IHC)
2. Gerdes J, *et al.* 1984. *J. Immunol.* 133:1710. (ICFC)
3. Schluter C, *et al.* 1993 *J. Cell Biol.* 123:513. (IHC, WB)
4. Bading H, *et al.* 1989 *Exp. Cell. Res.* 185:50. (IF)
5. Guha P, *et al.* 2013. *PNAS.* 110:5052. PubMed

Description:

Antigen Ki-67 is a nuclear protein expressed as two isoforms with molecular weights of 395 and 345 kD. Both isoforms contain one forkhead-associated domain and 16 concatenated "Ki-67 repeats," each containing the epitope recognized by the mAb Ki-67. The antigen Ki-67 interacts with Hklp2, hNIFK, and chromobox protein homolog 1, 3, and 5. Ki-67 is required for cell proliferation and its expression is restricted to the phases G_1, S, G_2, and M of the cell cycle. This characteristic makes Ki-67 an excellent marker for proliferating cells and is commonly used as one of the prognostic factors in cancer studies. Ki-67 has also been used to study myocyte proliferation after myocardial infarction as well as lymphocyte proliferation during infection, and has been used in neurons of patients with different neuropathologies.

Antigen References:

1. Byeon IJ, *et al.* 2005. *Nat. Struct. Mol. Biol.* 12:987.
2. Yerushalmi R, *et al.* 2010. *Lancet. Oncol.* 11:174.
3. Beltrami AP, *et al.* 2001. *N. Engl. J. Med.* 344:1750.
4. Sachsenber