Alexa Fluor® 647 anti-human Ki-67 Antibody

Catalog# / Size	350509 / 25 tests 350510 / 100 tests
Clone	Ki-67
Regulatory Status	RUO
Other Names	Antigen Ki-67
Isotype	Mouse IgG1, κ
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.

Product Details

Verified Reactivity	Human
Reported Reactivity	Cow
Antibody Type	Monoclonal
Host Species	Mouse
Immunogen	Nuclei of the Hodgkin lymphoma cell line L428
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA)
Preparation	The antibody was purified by affinity chromatography and conjugated with Alexa Fluor® 647 under optimal conditions.
Concentration	Lot-specific (to obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.)
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**
Application	ICFC - Quality tested ICC - Verified
Recommended Usage	Each lot of this antibody is quality control tested by our Ki-67 staining protocol below. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood. For immunocytochemistry, a concentration of 5.0 µg/ml is recommented. It is recommended that the reagent be titrated for optimal performance for each application. * Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633 nm / 635 nm. Alexa Fluor® and Pacific Blue™ are trademarks of Life Technologies Corporation. View full statement regarding label licenses
Excitation Laser	Red Laser (633 nm)
Application Notes	Additional reported applications (for the relevant formats) include: immunohistochemical staining of frozen tissue sections[1], Western blotting[3], and immunofluorescence microscopy[4]. **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 µl 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

(PubMed link indicates BioLegend citation)

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

Product Citations

1. Joshi SK, *et al.* 2021. Cancer Cell. . PubMed
2. Trabanelli S, *et al.* 2017. Nat Commun. 10.1038/s41467-017-00678-2. PubMed
3. Pei S *et al.* 2018. Cell stem cell. 23(1):86-100 . PubMed
4. Ong SM, *et al.* 2018. Cell Death Dis. 9:266. PubMed
5. Lindesmith LC, *et al.* 2020. Cell Mol Gastroenterol Hepatol. 0.586805556. PubMed
6. Lin JR *et al.* 2018. eLife. 7 pii: e31657. PubMed
7. Schulz E, *et al.* 2021. Toxins (Basel). 13:. PubMed
8. Yokomizo-Nakano T, *et al.* 2020. Cancer Res. 80:2523. PubMed
9. Watanabe N, *et al.* 2017. Oncoimmunology. 5:e1253656. PubMed

RRID

AB_10900810 (BioLegend Cat. No. 350509)
AB_10900821 (BioLegend Cat. No. 350510)

Antigen Details

Structure

Two isoforms with molecular weights of 395 and 345 kD, one forkhead-associated domain, 16 concatenated Ki-67 repeats, located in nucleus

Distribution

Expressed in the phases G_1, S, G_2, and M of the cell cycle

Function

Required for cell proliferation

Interaction

Chromobox protein homolog 1, 3 and 5, Hklp2, and hNIFK

Biology Area

Cell Biology, Cell Cycle/DNA Replication, DNA Repair/Replication

Molecular Family

Nuclear Markers

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. Sachsenberg N, *et al.* 1998. *J. Exp. Med.* 187:1295.
5. Nagy Z, *et al.* 1997. *Acta. Neuropathol.* 93:294.

Gene ID

4288

Related Protocols

Immunocytochemistry Staining Protocol

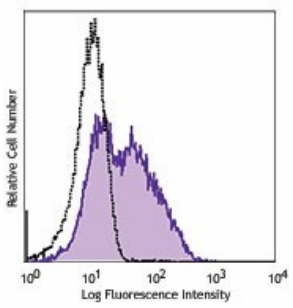
Ki-67 Flow Cytometry Staining Protocol

Other Formats

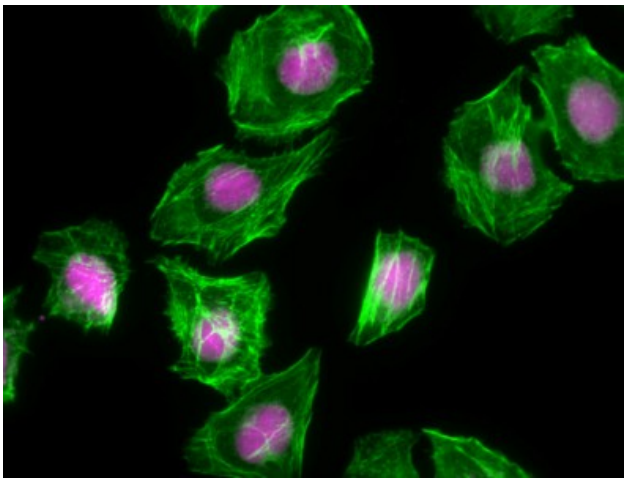
Brilliant Violet 510™ anti-human Ki-67, Purified anti-human Ki-67, PE anti-human Ki-67, Brilliant Violet 421™ anti-human Ki-67, Alexa Fluor® 488 anti-human Ki-67, Alexa Fluor® 647 anti-human Ki-67, Pacific Blue™ anti-human Ki-67, APC anti-human Ki-67, Brilliant Violet 711™ anti-human Ki-67, PerCP/Cyanine5.5 anti-human Ki-67, Brilliant Violet 605™ anti-human Ki-67, PE/Cyanine7 anti-human Ki-67, Purified anti-human Ki-67 (Maxpar® Ready), Alexa Fluor® 594 anti-human Ki-67, Alexa Fluor® 700 anti-human Ki-

67, PE/Dazzle™ 594 anti-human Ki-67, Brilliant Violet 750™ anti-human Ki-67

Product Data



PHA-stimulated (3 days) human peripheral blood lymphocytes were fixed and permeabilized with 70% ethanol, and then stained with Ki-67 Alexa Fluor® 647 (filled histogram) or mouse IgG1 κ Alexa Fluor® 647 isotype control (open histogram).



HeLa cells were fixed with 1% paraformaldehyde (PFA) for 10 minutes, permeabilized with 0.5% Triton X-100 for 10 minutes, and blocked with 5% FBS for 30 minutes. The cells were then intracellularly stained with 5 µg/ml of Ki-67 (clone Ki-67) Alexa Fluor® 647 (red) in blocking buffer overnight at 4°C and followed by Alexa Fluor® 488 Phalloidin (green) staining for 20 minutes. Nuclei were counterstained with DAPI and are shown in blue. The image was captured with 40X objective.

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