Purified anti-human Ki-67 Antibody

Catalog# / Size	350501 / 25 µg 350502 / 100 µg
Clone	Ki-67
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

Reactivity	Human, **Cross-Reactivity:** Bovine
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.
Preparation	The antibody was purified by affinity chromatography.
Concentration	0.5 mg/ml
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C.
Application	ICFC - *Quality tested* CyTOF®, ICC, WB - *Validated* IHC-F - *Reported in the literature*
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 ≤ 0.25 µg per million cells in 100 µl volume. For Western blotting, the suggested use of this reagent is 0.5 µg per ml (1:1000). It is recommended that the reagent be titrated for optimal performance for each application.
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. Guha P, et al. 2013. Proc Natl Acad Sci U S A. 110:5052. PubMed

2. Santos R, et al. 2017. Nat Commun. . 10.1038/s41467-017-01760-5. PubMed
3. Xiong Y, et al. 2019. Onco Targets Ther. 12:993. PubMed
4. Vining KH, et al. 2018. Adv Mater. 30:4. PubMed
5. Wang Z et al. 2019. Br J Pharmacol. 176(17):3390-3406 . PubMed
6. Bruno TC, et al. 2017. Cancer Immunol Res. 0.831944444. PubMed
7. Agrawal N, et al. 2018. Front Immunol. 2.053472222. PubMed
8. Eccles JD, et al. 2020. Cell Rep. 30:351. PubMed

RRID

AB_10662749 (BioLegend Cat. No. 350501)
AB_10662385 (BioLegend Cat. No. 350502)

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

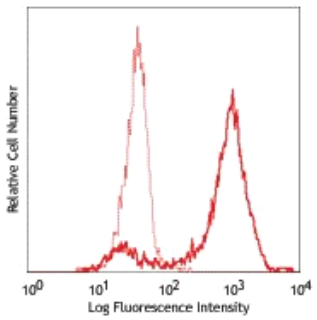
Immunocytochemistry Staining Protocol

Ki-67 Flow Cytometry Staining Protocol

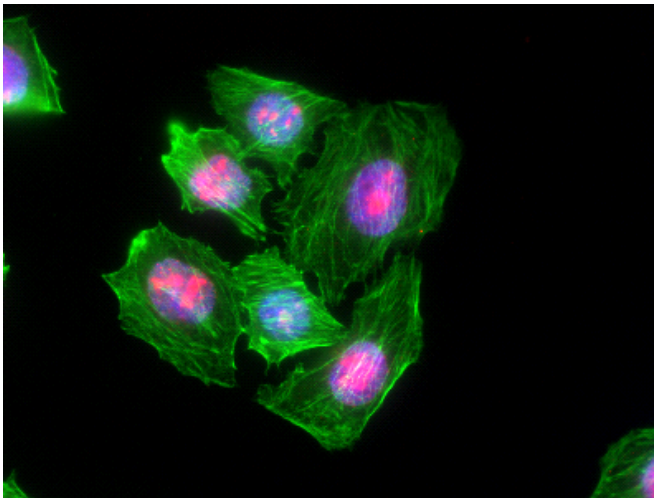
Other Formats

Brilliant Violet 510™ anti-human Ki-67, 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

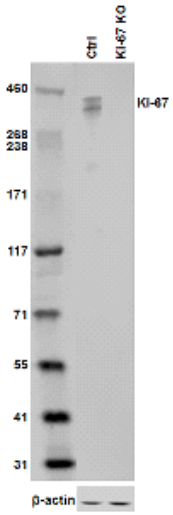
Product Data



Resting (dashed line) or PHA-activated human peripheral blood lymphocytes (day-3, solid line) fixed and permeabilized with 70% ethanol, then intracellularly stained with Ki-67 PE.



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 2.5 µg/ml of purified Ki-67 (clone Ki-67) (red) in blocking buffer overnight at 4°C and followed by DyLight™ 594 anti-mouse IgG and 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.



Total lysates (15 µg protein) from 293T control (Ctrl) and Ki-67 CRISPR/Cas9 knock out (Ki-67 KO) cells were resolved by electrophoresis (4-20% Tris-glycine gel), transferred to nitrocellulose, and probed with 1:1000 (0.5 µg/ml) purified anti-Ki-67 antibody, clone Ki-67. Proteins were visualized using chemiluminescence detection by incubation with HRP Goat anti-Mouse secondary antibody (Cat. No. 405306, 1:3000 dilution). Direct-Blot™ HRP anti-β-actin antibody was used as a loading control (Cat. No. 643807, 1:8000 dilution).

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