BioLegend
The path to legendary discovery™

Product Data Sheet

Alexa Fluor® 647 anti-human Ki-67

Catalog # / Size: 350509 / 25 tests
350510 / 100 tests

Clone: Ki-67

Isotype: Mouse IgG1, κ

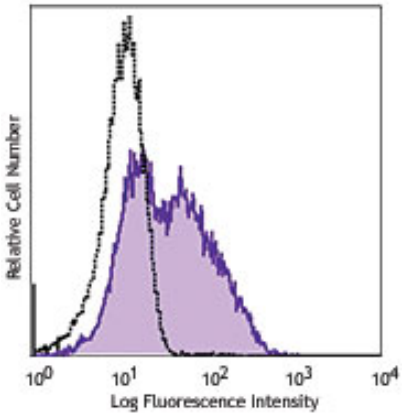
Immunogen: Nuclei of the Hodgkin lymphoma cell line L428

Reactivity: Human, **Cross-Reactivity:** Bovine

Preparation: The antibody was purified by affinity chromatography, and conjugated with Alexa Fluor® 647 under optimal conditions.

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

Storage: The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**



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).

Applications:

Applications: ICFC - *Quality tested*
IF - *Validated*

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 or 5 µl per 100 µl of whole blood. 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.

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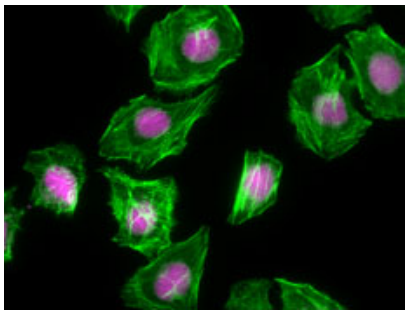

HeLa cells were fixed with 1% paraformaldehyde (PFA) for 10 minutes, permeabilized with 0.5% Triton X-100 for 10 minutes, and blocked with 5% FBS for 30 minutes. The cells were then intracellularly stained with 5 µg/ml of Ki-67 (clone Ki-67) Alexa Fluor® 647 (red) in blocking buffer overnight at 4°C 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.

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:

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.

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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.

Related Products:

Product	Clone	Application
Alexa Fluor® 647 Mouse IgG1, κ Isotype Ctrl (FC)	MOPC-21	FC, IF
Cell Staining Buffer		FC, ICC, ICFC
RBC Lysis Buffer (10X)		FC, ICFC
Human TruStain FcX™ (Fc Receptor Blocking Solution)		FC, ICC, ICFC

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