

## Alexa Fluor<sup>®</sup> 594 anti-human Ki-67 Antibody

<b>Catalog# / Size</b>	350528 / 100 µg
<b>Clone</b>	Ki-67
<b>Regulatory Status</b>	RUO
<b>Other Names</b>	Antigen Ki-67
<b>Isotype</b>	Mouse IgG1, κ
<b>Description</b>	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 <sub>1</sub> , S, G <sub>2</sub> , 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

---

<b>Verified Reactivity</b>	Human
<b>Reported Reactivity</b>	Cow
<b>Antibody Type</b>	Monoclonal
<b>Host Species</b>	Mouse
<b>Immunogen</b>	Nuclei of the Hodgkin lymphoma cell line L428
<b>Formulation</b>	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
<b>Preparation</b>	The antibody was purified by affinity chromatography and conjugated with Alexa Fluor <sup>®</sup> 594 under optimal conditions.
<b>Concentration</b>	0.5 mg/ml
<b>Storage &amp; Handling</b>	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. <b>Do not freeze.</b>
<b>Application</b>	<a href="#">ICC - Quality tested</a>
<b>Recommended Usage</b>	Each lot of this antibody is quality control tested by immunocytochemistry. For immunocytochemistry, a concentration range of 1.0 - 5.0 µg/ml is recommended. It is recommended that the reagent be titrated for optimal performance for each application.

\* Alexa Fluor<sup>®</sup> 594 has an excitation maximum of 590 nm, and a maximum emission of 617 nm.

Alexa Fluor<sup>®</sup> and Pacific Blue™ are trademarks of Life Technologies Corporation.

[View full statement regarding label licenses](#)

**Application Notes** Additional reported applications (for the relevant formats) include: immunohistochemical staining of frozen tissue sections<sup>1</sup>, Western blotting<sup>3</sup>, and immunofluorescence microscopy<sup>4</sup>.

#### **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 350xg 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<sup>6</sup>/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. Vining KH, *et al.* 2018. *Adv Mater.* 30:4. [PubMed](#)

#### RRID

AB\_2563504 (BioLegend Cat. No. 350528)

## Antigen Details

---

<b>Structure</b>	Two isoforms with molecular weights of 395 and 345 kD, one forkhead-associated domain, 16 concatenated Ki-67 repeats, located in nucleus
<b>Distribution</b>	Expressed in the phases G <sub>1</sub> , S, G <sub>2</sub> , and M of the cell cycle
<b>Function</b>	Required for cell proliferation
<b>Interaction</b>	Chromobox protein homolog 1, 3 and 5, Hklp2, and hNIFK
<b>Biology Area</b>	Cell Biology, Cell Cycle/DNA Replication, DNA Repair/Replication
<b>Molecular Family</b>	Nuclear Markers
<b>Antigen References</b>	<ol style="list-style-type: none"><li>1. Byeon IJ, <i>et al.</i> 2005. <i>Nat. Struct. Mol. Biol.</i> 12:987.</li><li>2. Yerushalmi R, <i>et al.</i> 2010. <i>Lancet. Oncol.</i> 11:174.</li><li>3. Beltrami AP, <i>et al.</i> 2001. <i>N. Engl. J. Med.</i> 344:1750.</li><li>4. Sachsenberg N, <i>et al.</i> 1998. <i>J. Exp. Med.</i> 187:1295.</li><li>5. Nagy Z, <i>et al.</i> 1997. <i>Acta. Neuropathol.</i> 93:294.</li></ol>
<b>Gene ID</b>	<a href="#">4288</a>

## Related Protocols

---

[Immunocytochemistry Staining Protocol](#)

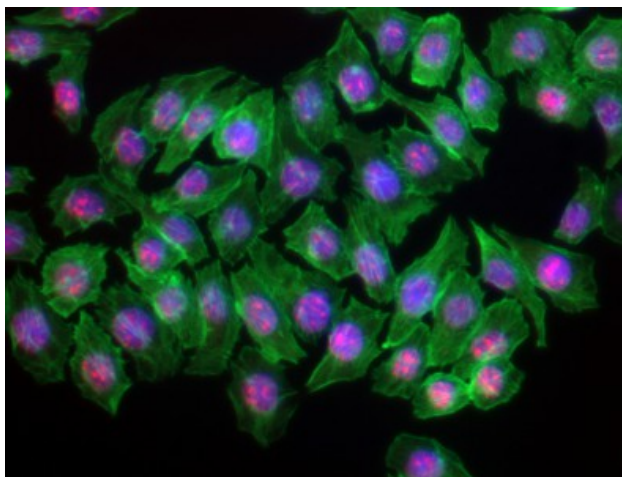
## Other Formats

---

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

---



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. Then the cells were intracellularly stained with 5  $\mu\text{g}/\text{ml}$  anti-human Ki-67 (clone Ki-67) Alexa Fluor® 594 (red) in blocking buffer overnight at 4°C and followed by Alexa Fluor® 488 Phalloidin (green) staining for 20 minutes at 4°C. Nuclei were counterstained with DAPI (blue). The image was captured with a 20X objective.

For research use only. Not for diagnostic use. Not for resale. BioLegend will not be held responsible for patent infringement or other violations that may occur with the use of our products.

\*These products may be covered by one or more Limited Use Label Licenses (see the BioLegend Catalog or our website, [www.biolegend.com/ordering#license](http://www.biolegend.com/ordering#license)). BioLegend products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products, reverse engineer functionally similar materials, or to provide a service to third parties without written approval of BioLegend. By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 [www.biolegend.com](http://www.biolegend.com)  
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587