

## Brilliant Violet 510™ anti-human Ki-67

**Catalog # / Size:** 2352585 / 25 tests  
2352590 / 100 tests

**Clone:** Ki-67

**Isotype:** Mouse IgG1,  $\kappa$

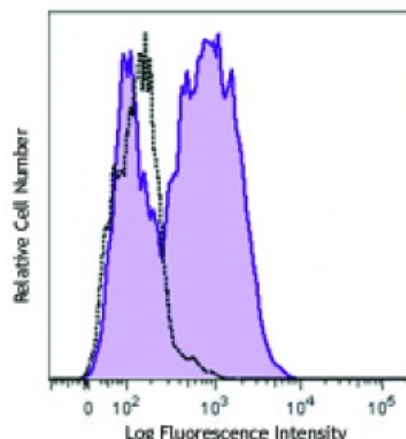
**Immunogen:** Nuclei of the Hodgkin lymphoma cell line L428

**Reactivity:** Human

**Preparation:** The antibody was purified by affinity chromatography and conjugated with Brilliant Violet 510™ under optimal conditions. The solution is free of unconjugated Brilliant Violet 510™ and unconjugated antibody.

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

**Concentration:** Lot-specific

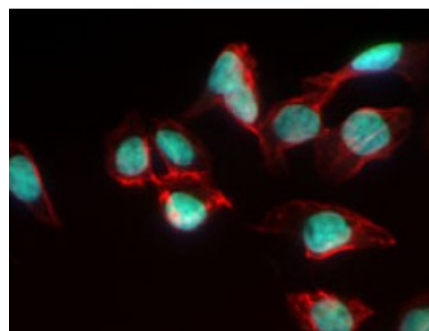


PHA-stimulated (3 days) human peripheral blood lymphocytes were fixed and permeabilized with 70% ethanol, and then stained with Ki-67 Brilliant Violet 510™ (filled histogram) or mouse IgG1  $\kappa$  Brilliant Violet 510™ isotype control (open histogram).

## 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, the suggested use of this reagent is  $\leq 5$   $\mu$ L per million cells or 5  $\mu$ L per 100  $\mu$ L of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.



Brilliant Violet 510™ excites at 405 nm and emits at 510 nm. The bandpass filter 510/50 nm is recommended for detection, although filter optimization may be required depending on other fluorophores used. **Be sure to verify that your cytometer configuration and software setup are appropriate for detecting this channel.** Refer to your instrument manual or manufacturer for support. Brilliant Violet 510™ is a trademark of Sirigen Group Ltd.

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  $\mu$ g/ml of Ki-67 (clone Ki-67) Brilliantia

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purposes only. This product may not be resold or incorporated in any manner into another product for resale. Any use for therapeutics or diagnostics is strictly prohibited. This product is covered by U.S. Patent(s), pending patent applications and foreign equivalents.

**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 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](#)

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**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<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.

**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