

Brilliant Violet 421™ anti-human Ki-67

Catalog # / Size: 350505 / 25 tests
350506 / 100 tests

Clone: Ki-67

Isotype: Mouse IgG1, κ

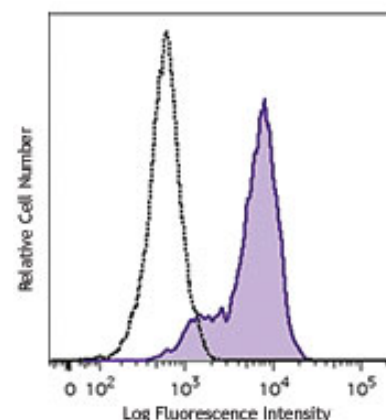
Immunogen: Nuclei of the Hodgkin lymphoma cell line L428

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

Preparation: The antibody was purified by affinity chromatography and conjugated with Brilliant Violet 421™ under optimal conditions. The solution is free of unconjugated Brilliant Violet 421™ and unconjugated antibody.

Formulation: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 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.**



3-day PHA-stimulated (top) or non-stimulated (bottom) human peripheral blood lymphocytes were fixed with 70% cold ethanol, then stained with Ki-67 Brilliant Violet 421™ (filled histogram) or mouse IgG1 Brilliant Violet 421™ isotype control (open histogram).

Applications:

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

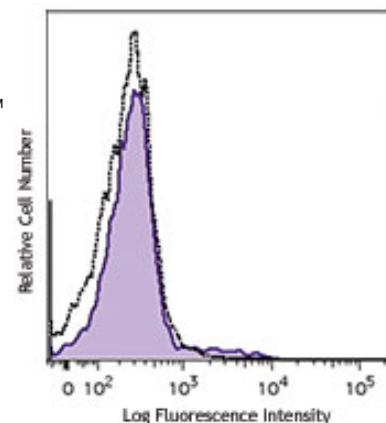
Recommended Usage: Each lot of this antibody is quality control tested by our Ki-67 staining protocol below. For flow cytometric staining, the suggested use of this reagent is $\leq 5 \mu\text{l}$ per million cells or $5 \mu\text{l}$ per $100 \mu\text{l}$ of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.

Brilliant Violet 421™ excites at 405 nm and emits at 421 nm. The standard bandpass filter 450/50 nm is recommended for detection. Brilliant Violet 421™ is a trademark of Sirigen Group Ltd.

Application Notes: Additional reported applications (for the relevant formats) include: immunohistochemical staining of frozen tissue sections¹, Western blotting³, and immunofluorescence microscopy⁴.

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 $0.5\text{-}10 \times 10^6/\text{ml}$.
7. Mix $100 \mu\text{l}$ 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 G₁, S, G₂, 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
	Brilliant Violet 421™ Mouse IgG1, κ	MOPC-21	FC, ICFC, IF
	Isotype Ctrl		
	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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