SONY

Product Data Sheet

Brilliant Violet 421™ anti-mouse Ki-67

Catalog # / Size: 3862055 / 50 µg

Clone: 16A8

Isotype: Rat IgG2a, κ

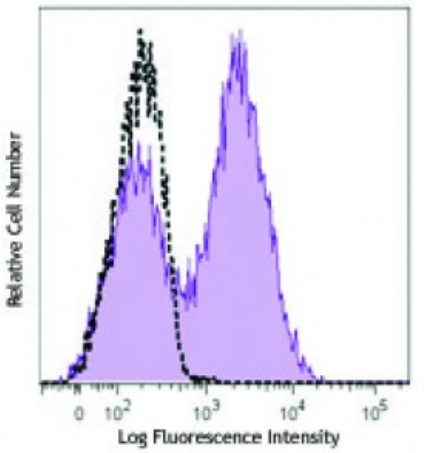
Immunogen: *E. coli* expressed partial mouse Ki-67 recombinant protein, 1816-2163 aa.

Reactivity: Human,Mouse

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).

Concentration: 0.2



Con A+IL-2-stimulated (3 days) C57BL/6 mouse splenocytes were fixed and permeabilized with 70% ethanol, and then stained with Ki-67 (clone 16A8) Brilliant Violet 421™ (filled histogram) or rat IgG2a, κ Brilliant Violet 421™ isotype contr

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 ≤0.25 microg per million cells in 100 microL volume. 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.

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Application Notes: Additional reported applications (for the relevant formats) include: immunofluorescence staining.

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 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 and then resuspend in 0.5 ml cell staining buffer for flow cytometric analysis.

Application References:

1. Medina-Reyes EI, *et al.* 2015. *Environ Res.* 136:424. PubMed
2. Guillaumond F, *et al.* 2015. *PNAS.* 112:2473. PubMed
3. Sharma SK, *et al.* 2015. *J Immunol.* 194:5529. PubMed
4. Rodero MP, *et al.* 2014. *J. Invest. Dermatol.* 7:1991-7. PubMed

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Description: The nuclear protein Ki-67 was first identified by the monoclonal antibody Ki-67, which was generated by immunizing mice with nuclei of the L428 Hodgkin lymphoma cell line. Ki-67 protein plays an essential role in ribosomal RNA transcription and cell proliferation. Expression of Ki-67 occurs during G1, S, G2, and M phase, while in G0 phase the Ki-67 protein is not detectable. Ki-67 is strongly expressed in proliferating cells and has been reported as a prognostic marker in various tumors.

Antigen References:

1. Starborg M, *et al.* 1996. *J. Cell. Sci.* 109:143.
2. Byeon IJ, *et al.* 2005. *Nat. Struct. Mol. Biol.* 12:987.
3. Yerushalmi R, *et al.* 2010. *Lancet. Oncol.* 11:174.
4. Beltrami AP, *e*