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Product Data Sheet

Alexa Fluor® 647 anti-mouse Ki-67

Catalog # / Size: 3862040 / 100 µg
3862035 / 25 µg

Clone: 16A8

Isotype: Rat IgG2a, κ

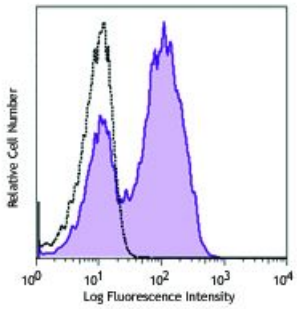
Immunogen: *E. coli* expressed partial mouse Ki-67 recombinant protein, 1816-2163 aa.

Reactivity: Mouse

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.

Concentration: 0.5



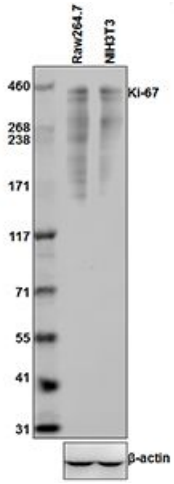
Con A-stimulated (3 days) BALB/c mouse splenocytes were fixed and permeabilized with 70% ethanol, then stained with Ki-67 (clone 16A8) Alexa Fluor® 647 (filled histogram) or rat IgG2a, κ Alexa Fluor® 647 isotype control (open histogram).

Applications:

Applications: Intracellular Staining for 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 µg per million cells in 100 µl volume. 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.



Total cell lysates (15 µg protein) from Raw264.7 and NIH3T3 were resolved by 3-8% Tris-Acetate gel electrophoresis, transferred to nitrocellulose, and probed with mouse Ki-67 antibody (clone 16A8). Proteins were visualized using a goat anti-rat IgG secondary antibody conjugated to HRP and chemiluminescence detection. Direct-Blot™ HRP anti-β-actin was used as a loading control.

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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 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 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, *et al.* 2001. *N. Engl. J. Med.* 344:1750.
5. Sachsenberg N, *et al.* 1998. *J. Exp. Med.* 187:1295.
6. Nagy Z, *et al.* 1997. *Acta. Neuropathol.* 93:294.

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