SONY

# Product Data Sheet

## Pacific Blue™ anti-mouse Ki-67

**Catalog # / Size:** 3862110 / 100 µg
3862105 / 25 µg

**Clone:** 16A8

**Isotype:** Rat IgG2a, κ

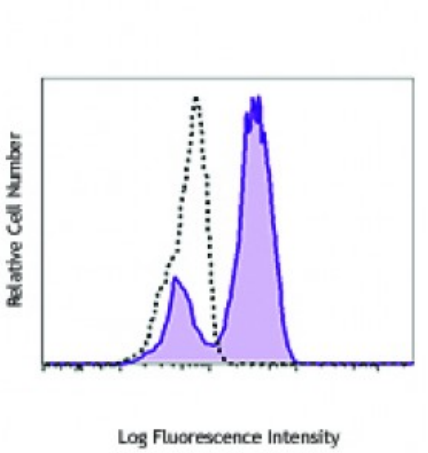
**Immunogen:** *E. coli* expressed partial mouse Ki-67 recombinant protein, 1816-2163 aa.

**Reactivity:** Human,Mouse

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

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

**Concentration:** 0.5




Con A-stimulated (three days) C57BL/6 mouse splenocytes were fixed and permeabilized with 70% ethanol, then stained with Ki-67 (clone 16A8) Pacific Blue™ (filled histogram) or rat IgG2a, κ Pacific Blue™ 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 ≤1.0 microg per million cells in 100 microL volume or 100 microL of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.

* Pacific Blue™ has a maximum emission of 455 nm when it is excited at 405 nm. Prior to using Pacific Blue™ conjugate for flow cytometric analysis, please verify your flow cytometer's capability of exciting and detecting the fluorochrome.

**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*