

## Pacific Blue™ anti-human Ki-67

**Catalog # / Size:** 350511 / 25 tests  
350512 / 100 tests

**Clone:** Ki-67

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

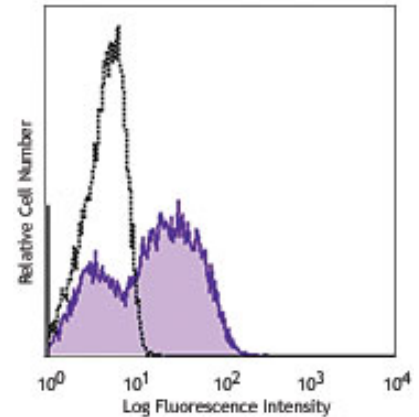
**Immunogen:** Nuclei of the Hodgkin lymphoma cell line L428

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

**Preparation:** The antibody was purified by affinity chromatography, and conjugated with 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 and 0.2% (w/v) 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.**



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

## Applications:

**Applications:** ICFC - *Quality tested*  
IF - *Reported in the literature*

**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 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.

\* 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.

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**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  $\mu$ 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 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.



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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.

<b>Related Products:</b>	<b>Product</b>	<b>Clone</b>	<b>Application</b>
	Pacific Blue™ Mouse IgG1, κ Isotype Ctrl	MOPC-21	FC, ICFC
	Cell Staining Buffer		FC, ICC, ICFC
	RBC Lysis Buffer (10X)		FC, ICFC
	Fixation Buffer		ICC, ICFC
	Permeabilization Wash Buffer (10X)		ICC, ICFC, IHC
	Human TruStain FcX™ (Fc Receptor Blocking Solution)		FC, ICC, ICFC

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