Technical Data Sheet

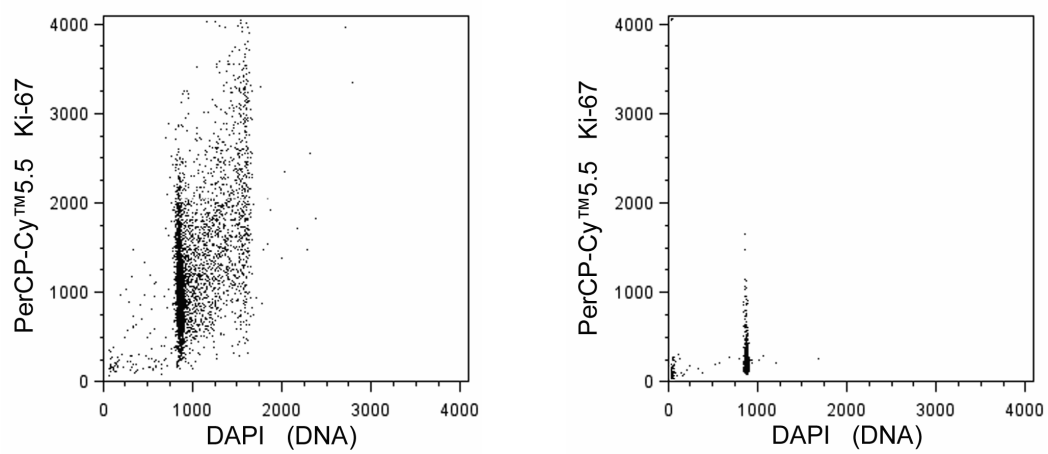
PerCP-Cy™5.5 Mouse anti-Human Ki-67

Product Information

Material Number:	**561284**
Alternate Name:	MKI67; Antigen identified by monoclonal antibody Ki-67; KIA
Size:	50 tests
Vol. per Test:	5 µl
Clone:	B56
Immunogen:	Human Ki-67
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Reported: Mouse, Rat, Chicken, Dog
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The B56 monoclonal antibody specifically binds to the Ki-67 antigen that is expressed in the nucleus of cycling cells (G1, S, G2, M cell cycle phases). During the G0 phase, the antigen cannot be detected. During interphase of the cell cycle, it is associated with nucleolar components, and it is on the surface of the chromosomes during M phase. Ki-67 is a large protein having 2 alternatively spliced isoforms, an N-terminal forkhead-associated domain, a C-terminal domain that binds to heterochromatin proteins, and multiple phosphorylation sites, the functions of which are still unclear. Because of the strict association of Ki-67 expression with cell proliferation, anti-Ki-67 antibodies are useful for the identification, quantification, and monitoring of growing cell populations.



Flow cytometric analysis of Ki-67 expression by proliferating Jurkat and noncycling human peripheral blood mononuclear cells (PBMC). *Jurkat and PBMC were fixed and permeabilized with 70% ice cold ethanol, washed, and stained with PerCP-Cy™5.5 Mouse anti-Human Ki-67 antibody (Cat. No. 561284) according to the BD Biosciences support protocol, Flow Cytometry Staining Protocol for Detection of Ki-67. The cells were then counterstained with 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma, Cat. No. D-9542) to stain double-stranded DNA. Two-color flow cytometric dot plots showing the correlated expression patterns of DAPI (DNA) staining versus Ki-67 were derived from gated events with the forward and side light-scatter characteristics of intact Jurkat cells (Left Panel) or PBMC (Right Panel). Flow cytometry was performed using a BD LSR™ II Flow Cytometer System.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
3. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
7. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
9. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
10. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Bruno S, Crissman HA, Bauer KD, Darzynkiewicz Z. Changes in cell nuclei during S phase: progressive chromatin condensation and altered expression of the proliferation-associated nuclear proteins Ki-67, cyclin (PCNA), p105, and p34. *Exp Cell Res.* 1991; 196(1):99-106. (Biology: Flow cytometry)

Bruno S, Darzynkiewicz Z. Cell cycle dependent expression and stability of the nuclear protein detected by Ki-67 antibody in HL-60 cells. *Cell Prolif.* 1992; 25(1):31-40. (Biology: Flow cytometry)

Byeon I-JL, Li H, Song H, Gronenborn AM, Tsai M-D. Sequential phosphorylation and multisite interactions characterize specific target recognition by the FHA domain of Ki67. *Nat Struct Mol Biol.* 2005; 12(11):987-993. (Biology)

Ho DWY, Fan ST, To J, et al. Selective plasma filtration for treatment of fulminant hepatic failure induced by D-galactosamine in a pig model. *Gut.* 2002; 50:869-876. (Clone-specific)

Kill IR. Localisation of the Ki-67 antigen within the nucleolus: evidence for a fibrillarin-deficient region of the dense fibrillar component. *J Cell Sci.* 1996; 109(6):1253-1263. (Biology)

Kouro T, Medina KL, Oritani K, Kincade PW. Characteristics of early murine B-lymphocyte precursors and their direct sensitivity to negative regulators. *Blood.* 2001; 97(9):2708-2715. (Clone-specific: Flow cytometry)

Scholzen T, Endl E, Wohlenberg, et al. The Ki-67 protein interacts with members of the heterochromatin protein 1 (HP1) family: a potential role in the regulation of higher-order chromatin structure. *J Pathol.* 2001; 196(2):135-144. (Biology)

Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol.* 2000; 182(3):311-322. (Biology)

Spargo LDJ, Cleland LG, Cockshell MP, Mayrhofer Graham. Recruitment and proliferation of CD4+ T cells in synovium following adoptive transfer of adjuvant-induced arthritis. *Int Immunol.* 2006; 18(6):897-910. (Clone-specific)

Starborg M, Gell K, Brundell E, Höög C. The murine Ki-67 cell proliferation antigen accumulates in the nucleolar and heterochromatic regions of interphase cells and at the periphery of the mitotic chromosomes in a process essential for cell cycle progression. *J Cell Sci.* 1996; 109(1):143-153. (Biology)