Technical Data Sheet

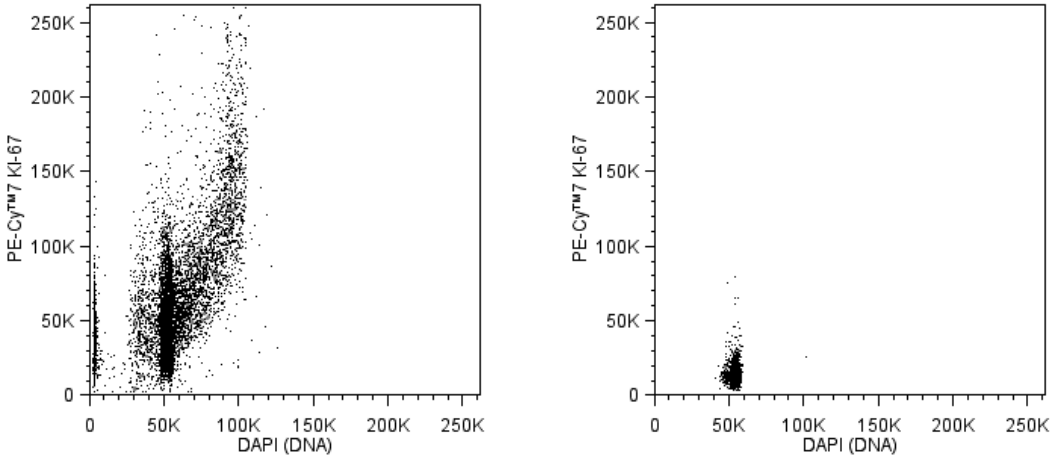
PE-Cy™7 Mouse anti-Human Ki-67

Product Information

Material Number:	**561283**
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.



Multicolor flow cytometric analysis of Ki-67 expression by proliferating Molt-4 and noncycling human peripheral blood mononuclear cells (PBMC). *Proliferating Molt-4 cells and noncycling PBMC were fixed and permeabilized with 70% ice cold ethanol, washed, and stained with PE-Cy™7 Mouse Anti-Human Ki-67 antibody (Cat. No. 561283) 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 Molt-4 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 PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

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

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Recommended Assay Procedure:

For more information, please refer to the Flow Cytometry Staining Protocol for Detection of Ki-67
http://www.bdbiosciences.com/support/resources/protocols/detection_ki_67.jsp#search=(Ki-67)

Suggested Companion Products

Catalog Number	Name	Size	Clone
556463	Propidium Iodide Staining Solution	2.0 ml	(none)
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. An isotype control should be used at the same concentration as the antibody of interest.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. 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.
7. 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.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
9. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
10. 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.
11. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.

References

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