

## Technical Data Sheet

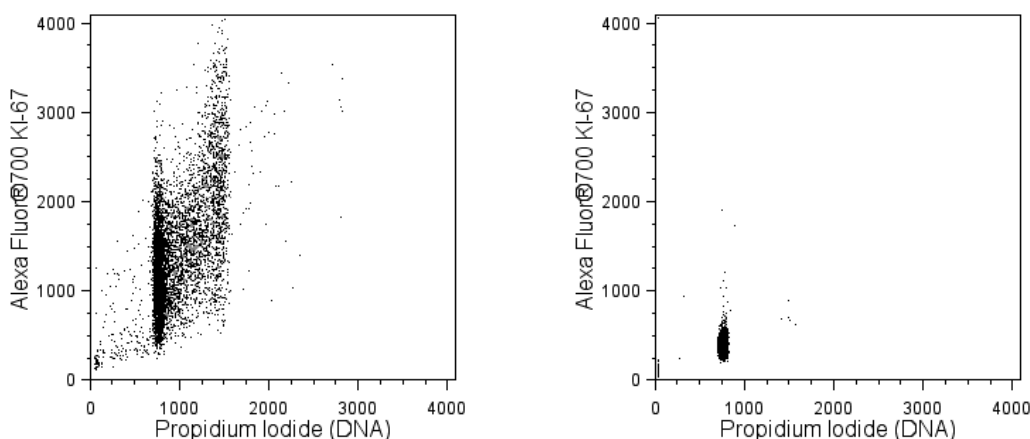
## Alexa Fluor® 700 Mouse anti-Human Ki-67

## Product Information

Material Number:	561277
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 protein stabilizer 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).** Human Jurkat and PBMC were fixed and permeabilized with 70% ice cold ethanol, washed, and stained with Alexa Fluor® 700 Mouse anti-Human Ki-67 antibody (Cat. No. 561277) according to the BD Biosciences support protocol, Flow Cytometry Staining Protocol for Detection of Ki-67. The cells were then RNase A (Sigma Cat. No. R-5500) treated and counterstained with Propidium Iodide Staining Solution (Cat. No. 556463) to stain double-stranded DNA. Two-color flow cytometric dot plots showing the correlated expression patterns of Propidium Iodide (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 to Alexa Fluor® 700 under optimum conditions, and unreacted Alexa Fluor® 700 was removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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## Application Notes

### Application

Intracellular staining (flow cytometry)

Routinely Tested

### 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  $1 \times 10^6$  cells in a 100- $\mu$ l experimental sample (a test).
2. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.
3. The Alexa Fluor<sup>®</sup>, Pacific Blue<sup>™</sup>, and Cascade Blue<sup>®</sup> dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor<sup>®</sup> dyes (except for Alexa Fluor<sup>®</sup> 430), Pacific Blue<sup>™</sup> dye, and Cascade Blue<sup>®</sup> dye are covered by pending and issued patents.
4. Alexa Fluor<sup>®</sup> 700 has an adsorption maximum of ~700nm and a peak fluorescence emission of ~720nm. Before staining cells with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. Alexa Fluor<sup>®</sup> is a registered trademark of Molecular Probes, Inc., Eugene, OR.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
8. An isotype control should be used at the same concentration as the antibody of interest.

### 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 fibrillar-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)