Technical Data Sheet

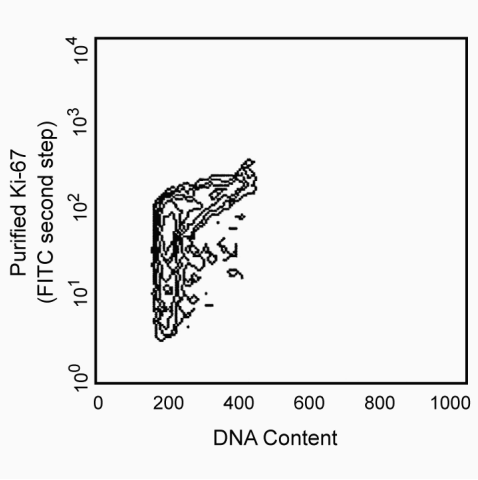
Purified Mouse Anti-Human Ki-67

Product Information

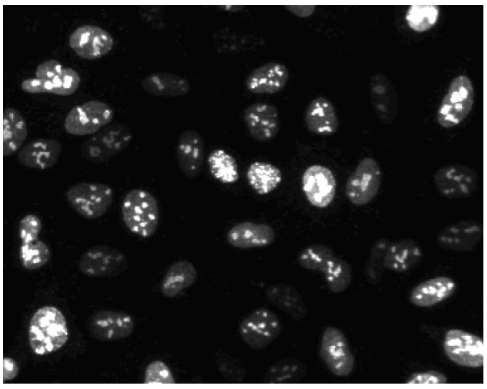
Material Number:	**556003**
Alternate Name:	MKI67; Antigen identified by monoclonal antibody Ki-67; KIA
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	B56
Immunogen:	Human Ki-67
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat, Pig
Storage Buffer:	Aqueous buffered solution containing ≤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.



Profile of peripheral MOLT-4 cells analyzed on a FACScan (BDIS, San Jose, CA)



Immunofluorescent staining of HeLa cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (below) and the anti-Ki67 antibody. The second step reagent was Alexa Fluor® 555 (Molecular Probes). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells and worked with both the Triton™ X-100 and methanol fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Bioimaging	Routinely Tested
Western blot	Not Recommended

BD Biosciences

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Recommended Assay Procedure:

STAINING PROTOCOL FOR FLOW CYTOMETRY:

1. Harvest, count and pellet cells following standard procedures (Note: Ki-67 is expressed by the proliferative cells. You may get no staining with the resting cells, e.g., unstimulated PBMC).
2. While votexing, add 5 ml drop by drop of cold 70-80% ethanol into the cells pellet (1-5x10^7 cells). Then incubate at -20°C for 2 hours minimum. These fixed cells can be used up to 60 days after fixing (store at -20°C).
3. Add 30-40 ml wash buffer (PBS with 1% FBS, 0.09% NaN3, pH 7.2) to the fixed cells. Centrifuge the cells for 10 minutes at 1000 rpm and aspirate supernatant. Wash one more time with 30-40 ml wash buffer. Centrifuge at 1000 rpm for 10 minutes and aspirate supernatant.
4. Resuspend the cells to a concentration of 1 x 10^7/ml (1 x 10^6/100 µl).
5. Transfer 100 µl cell suspension into each fresh tube.
6. Add 20 µl of properly diluted antibody according to the protocol into the tubes above. Mix gently.
7. Incubate the tubes at room temperature (RT) for 20-30 minutes in the dark.
8. Wash with 2 ml of PBS washing buffer at 1000 rpm for 5 minutes.
9. Aspirate the supernatant.
10. For direct conjugated antibody: go to steps 13 & 14.
11. For purified antibody: add 50 µl of diluted secondary antibody at optimal concentration (Cat. No. 555988), incubate at RT for 30 minutes in the dark.
12. Repeat step 8 & 9.
13. Add 0.5 ml of PBS wash buffer into each tube. For FITC conjugated antibody, add µl of PI Staining Solution (Cat. No. 556463); for PE-conjugated antibody, add 20 µl BD Via-Probe™ Cell Viability Solution (Cat. No. 555816) into each tube.
14. Analyze the sample with FACS.

STAINING PROTOCOL FOR BIOIMAGING:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton™ X-100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555746	Purified Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
556463	Propidium Iodide Staining Solution	2.0 ml	(none)
555816	Cell Viability Solution	100 tests	(none)
555988	FITC Goat Anti-Mouse IgG/IgM	0.5 mg	Polyclonal
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.
4. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
5. An isotype control should be used at the same concentration as the antibody of interest.
6. Triton is a trademark of the Dow Chemical Company.

References

Kubbutat MH, Key G, Duchrow M, Schluter C, Flad HD, Gerdes J. Epitope analysis of antibodies recognising the cell proliferation associated nuclear antigen previously defined by the antibody Ki-67 (Ki-67 protein). *J Clin Pathol.* 1994; 47(6):524-528. (Biology)

Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem.* 1991; 39(6):741-748. (Biology)