BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

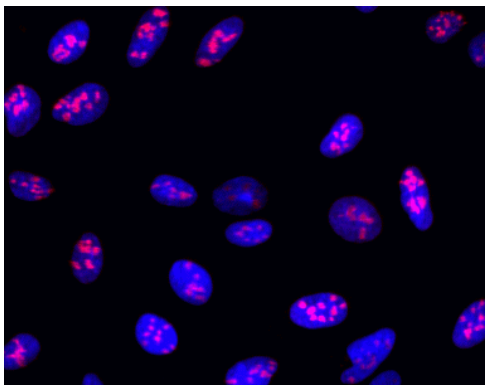
Alexa Fluor® 488 Mouse anti-Human Ki-67

Product Information

Material Number:	**558616**
Alternate Name:	MKI67; Antigen identified by monoclonal antibody Ki-67; KIA
Size:	100 tests
Vol. per Test:	5 µl
Clone:	B56
Immunogen:	Human Ki-67
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Reported Reactivity: Mouse, Rat, Pig
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.



Immunofluorescent staining of human cell lines. *U-2 OS cells (ATCC HTB-96) were cultured, fixed, permeabilized with cold methanol, stained with Alexa Fluor® 488 Mouse anti-Human Ki-67 (pseudo-colored red, which appears pink when co-localized with the blue) and counter-stained with Hoechst 33342 (pseudo-colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 855 Bioimager System with a 20x objective and merged using BD Attovision™ software. This antibody also stains A549 (ATCC CCL-185) and HeLa (ATCC CCL-2) cells, and it works with either cold methanol or Triton X-100 permeabilization (see Recommended Assay Procedure).*

Preparation and Storage

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

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

The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

Application Notes

Application

Bioimaging	Routinely Tested

Recommended Assay Procedure:

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either cold methanol or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or -20°C BD Phosflow™ Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.

   Triton is a trademark of The Dow Chemical Company.
4. Remove the permeabilizer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of blocking buffer (3% FBS in 1× PBS) or BD Pharmingen™ Stain Buffer (FBS)

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(Cat. No. 554656) to each well and incubating for 30 minutes at RT.

6. Remove the blocking buffer, dilute the antibody conjugate 1:10 in blocking buffer or Stain Buffer (FBS), and stain the cells by adding 50 µl of the diluted antibody conjugate to each well and incubating for 1 hour at RT.
7. Remove the diluted antibody conjugate, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
9. View and analyze the cells on an appropriate imaging instrument.

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate		(none)
554655	Fixation Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test when following the Recommended Assay Procedure. A Test is typically ~10,000 cells cultured in a well of a 96-well imaging plate.
2. 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.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® 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® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
7. Triton is a trademark of the Dow Chemical Company.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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