Technical Data Sheet

Purified Mouse Anti-Human Ki-67

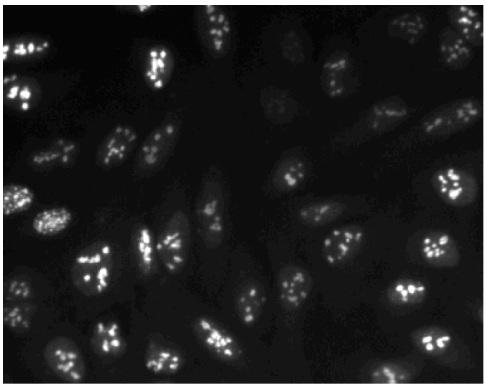
Product Information

Material Number:	**610969**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	35/Ki-67
Immunogen:	Human Ki-67 aa. 1547-1742
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	395 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Cellular proliferation is a complex multi-faceted process, central to biological events ranging from embryonic development to wound healing. Although proliferation is tightly controlled, dysregulation often results in tumorigenesis. The regulatory process involves the expression of many cell-cycle-associated proteins that are subject to cycle-dependent modification. Such protein expression may be monitored to assess the proliferative capacity of cells, especially tumorigenic cells.The Ki-67 antigen is a nuclear protein expressed exclusively in proliferating cells during all active parts of the cell cycle. However, it is absent in quiescent cells and during DNA repair. The distribution of Ki-67 changes during different stages of the cell cycle. During G1, it is localized to the perinuclear region, but is primarily found in the nuclear matrix in later phases. During mitosis, it associates with the condensed chromosomes. The nuclear localization of Ki-67 and strict association with the cell cycle indicate its importance in the regulation of cell division. Therefore, Ki-67 has become an important marker of proliferating cells, and may also be a marker for disitinct nuclear matrix compartments.

Western blot analysis of Ki-67 on a HeLa lysate (Cat. No. 611449). *Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of the Ki-67 antibody.*



Immunofluorescent staining of U-2 OS (ATCC HTB-96) 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 Triton™ X-100 perm protocol and the anti-Ki-67 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained HeLa (ATCC CCL-2) and A549 (ATCC CCL-185) cells and can be used with either perm protocol (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

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

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

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 BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or 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.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611449	HeLa Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(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. 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.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

References

Saitoh H, Pizzi MD, Wang J. Perturbation of SUMOlation enzyme Ubc9 by distinct domain within nucleoporin RanBP2/Nup358. *J Biol Chem.* 2002; 277(7):4755-4763. (Clone-specific: Immunofluorescence)

Schluter C, Duchrow M, Wohlenberg C, et al. The cell proliferation-associated antigen of antibody Ki-67: a very large, ubiquitous nuclear protein with numerous repeated elements, representing a new kind of cell cycle-maintaining proteins. *J Cell Biol.* 1993; 123(3):513-522. (Biology)

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)