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Product datasheet for TA336650

Ki67 (MKI67) Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	FC, ICC/IF, IHC, Immunoblotting, IP, WB
Recommended Dilution:	Flow Cytometry, Immunoblotting, Immunohistochemistry-Frozen: 1:50-1:300, Immunoprecipitation, Immunocytochemistry/ Immunofluorescence: 1:20-1:100, Western Blot: 1-2 ug/ml, Immunohistochemistry-Paraffin: 1:50-1:300, Knockout Validated, Immunohistochemistry: 1:50-1:300
Reactivity:	Human, Mouse, Rat, Porcine
Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Immunogen:	A synthetic peptide made to an internal region of human Ki67 (within residues 1550-1700) [Swiss-Prot# P46013]
Formulation:	PBS, 0.05% Sodium Azide. Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Concentration:	lot specific
Purification:	Immunogen affinity purified
Conjugation:	Unconjugated
Storage:	Store at -20°C as received.
Stability:	Stable for 12 months from date of receipt.
Gene Name:	marker of proliferation Ki-67
Database Link:	NP_001139438 Entrez Gene 17345 MouseEntrez Gene 291234 RatEntrez Gene 4288 Human P46013

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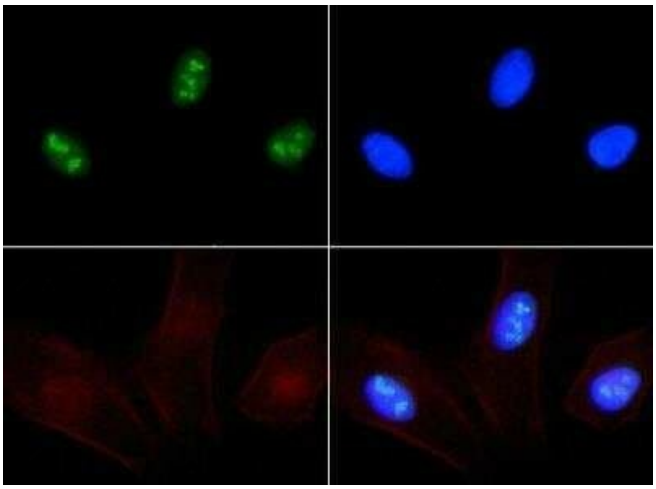
Background: Originally discovered employing mouse monoclonal antibody against a nuclear antigen from Hodgkin's lymphoma-derived cell line, this non-histone protein was named Ki67 after researcher's location (Gerdes and colleagues), Ki for Kiel University in Germany and 67 referring to the clone number on the 96-well plate. It interacts with KIF15 as well as MKI67IP, and is suggested to be involved in cell cycle regulation. Ki67 is a large protein with expected molecular weight of about 395 kD and has a very complex localization pattern within the nucleus, one which changes during cell cycle progression. Its expression occurs specially during late G1, S, G2 and M phases of the cell cycle, while in cells undergoing G0 phase, Ki67 remains undetectable. Ki67 undergoes phosphorylation/dephosphorylation during mitosis, is susceptible to proteases and its structure implies that its expression is regulated by proteolytic pathways. Ki67 is associated with nucleolar DFC (dense fibrillary component) and its regulation appears to be tightly controlled (estimated half life is 60-90 min, regardless of the cell position in the cell cycle), presumably by precise synthesis and degradation systems involving proteasome, a protease complex. Due to its association with cell divison process, Ki-67 is routinely used as cellular proliferation marker of solid tumors as well as certain hematological malignancies, and a correlation has been demonstrated between Ki-67 index and the histopathological grade of cancers.

Synonyms: KIA; MIB-; MIB-1; PPP1R105

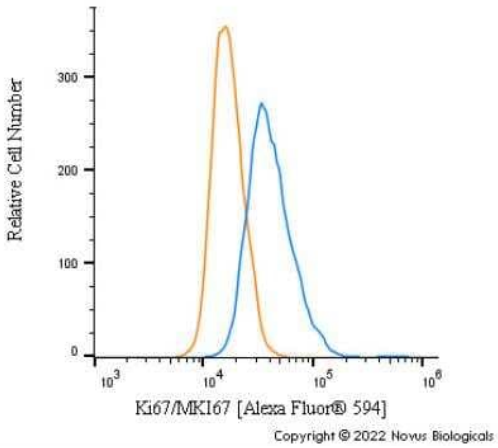
Note: This Ki67 antibody is useful for Immunohistochemistry on frozen and paraffin-embedded sections and Immunocytochemistry/Immunofluorescence. There have been mixed results in Western blot; however, NB500-170 has been used successfully Western Blog reported by a customer review. *Formalin fixed paraffin embedded tissue sections require high temperature antigen unmasking with 10 mM citrate buffer, pH 6.0 prior to immunostaining. This antibody will not work without optimal antigen retrieval. This is probably the most critical step. NOTE: We suggest an incubation period of 30 minutes at room temperature and to use DAB to stain the protein (immunofluorescence may give problems as the protein is nuclear).

Protein Families: Druggable Genome, ES Cell Differentiation/IPS

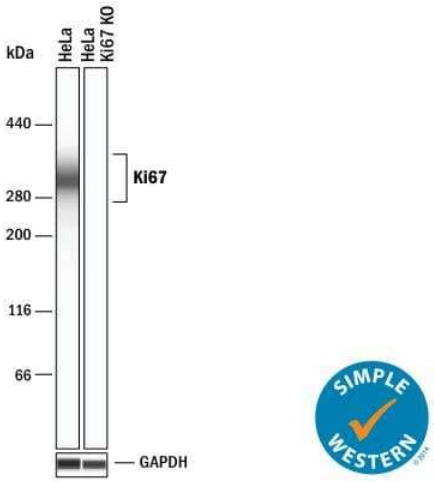
Product images:



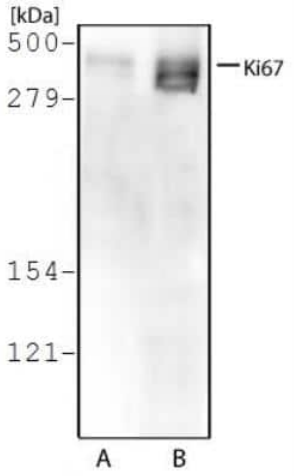
Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody TA336650 - Ki67 antibody was tested at 1:25 in HeLa cells with FITC (green). Nuclei and actin were counterstained with Dapi (blue) and Phalloidin (red).



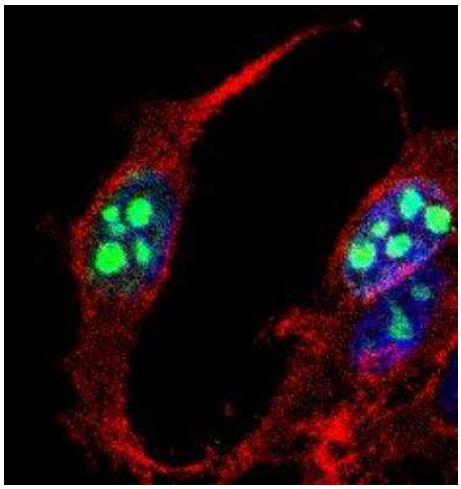
Flow Cytometry: Ki67/MKI67 Antibody - BSA Free TA336650 - An intracellular stain was performed on U-251 MG cells with Ki67/MKI67 Antibody TA336650AF594 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 594.



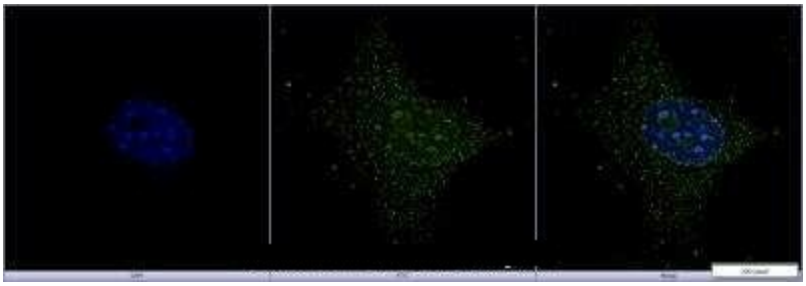
Knockout Validated: Ki67/MKI67 Antibody TA336650 - Detection of Ki67/MKI67 by Simple WesternTM. Simple Western lane view shows lysates of HeLa parental cell line and Ki67 knockout (KO) HeLa cell line. A specific band was detected for Ki67/MKI67 at approximately 320 kDa (as indicated) in the parental cell line, but is not detectable in the knockout HeLa cell line using 20 ug/mL of Rabbit Anti-Ki67/MKI67 Polyclonal Antibody (Catalog # TA336650). GAPDH is shown as a loading control. This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



Western Blot: Ki67/MKI67 Antibody TA336650 - Ki-67/MKI67 Antibody TA336650 - Analysis of A431 (A) and Hek293 (B) cell lysate using Ki67 antibody (TA336650) at 2 ug/ml.

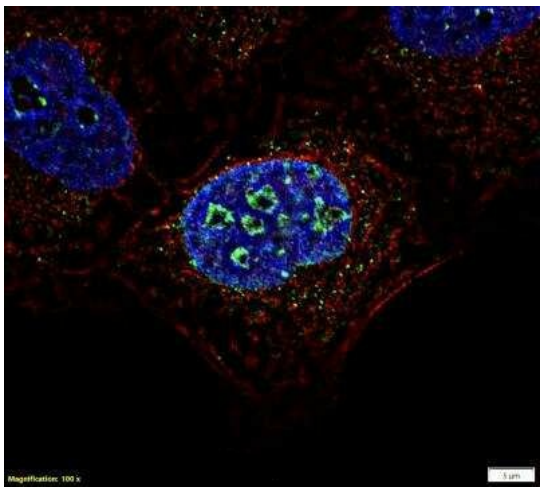


Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody TA336650 - Confocal immunofluorescent analysis of MCF7 cells using Ki67 antibody (TA336650, 1:5). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red). DAPI was used to stain the cell nuclei (blue).

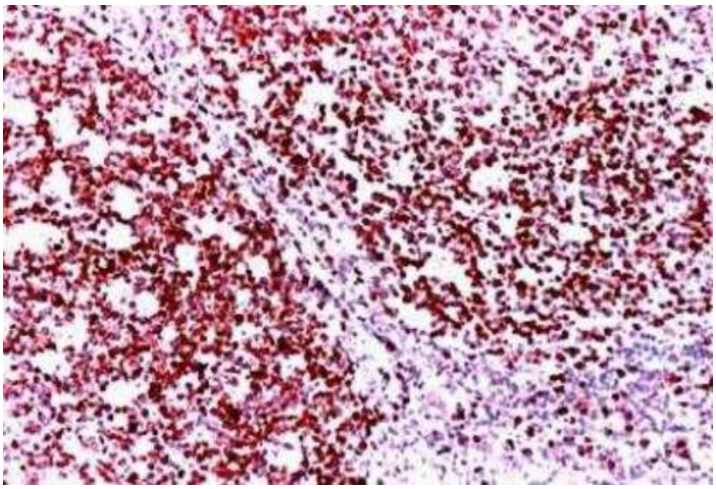


Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody TA336650 - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-Ki67/MKI67 Antibody TA336650 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.

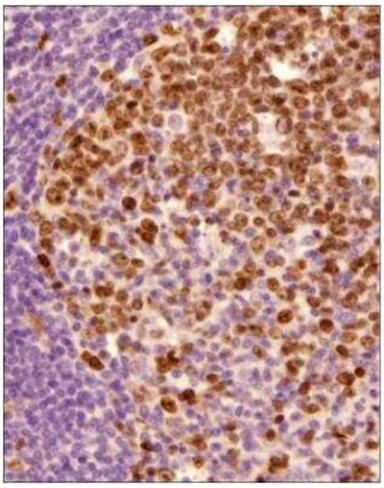
Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody TA336650 - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-TA336650 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:1000 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



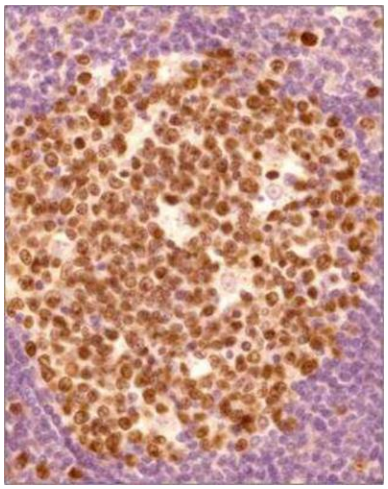
Immunocytochemistry/Immunofluorescence: Ki67/MKI67 Antibody TA336650 - A431 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-TA336650 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:1000 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



Immunohistochemistry-Paraffin: Ki67/MKI67 Antibody TA336650 - Ki-67/MKI67 Antibody TA336650 - Human tonsil.



Immunohistochemistry-Paraffin: Ki67/MKI67 Antibody TA336650 - Ki-67/MKI67 Antibody TA336650 - Human tonsil using 1:200 dilution of rabbit anti-KI67 antibody. The staining was developed with HRP labeled anti-rabbit IgG secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. This Ki67 antibody generated a specific nuclear staining in the cells in germinal centers of the tested tonsil tissue.



Immunohistochemistry-Paraffin: Ki67/MKI67 Antibody TA336650 - Ki-67/MKI67 Antibody TA336650 - Tissue section of human tonsil using 1:50 dilution of rabbit anti-KI67 antibody. The staining was developed with HRP labeled anti-rabbit IgG secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. This Ki67 antibody generated a specific nuclear staining in the cells in germinal centers of the tested tonsil tissue.