abcam

ab209897

Anti-Ki67 antibody [EPR3610] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR3610
Purification technique	Affinity purification Protein A
Concentration	1.033 - 1.036 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	-

Notes	If fixing cells in 4% PFA, it is recommended to permeabilized cells with 0.1% Triton-X for 5 min.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	**For unpurified use at 1/500 - 1/1000.**

Not recommended

Species	Mouse
Dilution info	-
Notes	**For unpurified use at 1/500 - 1/1000.**

Species	Rat
Dilution info	-
Notes	**For unpurified use at 1/500 - 1/1000.**

IHC-P

Tested

Species	Human
Dilution info	-
Notes	**For unpurified use at 1/500 - 1/1000.**

Not recommended

Species	Mouse
Dilution info	-
Notes	**For unpurified use at 1/500 - 1/1000.**

Species	Rat
Dilution info	-
Notes	**For unpurified use at 1/500 - 1/1000.**

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information MKI67

Function Protein that associates with the surface of mitotic chromosomes and acts both as a chromosome repellent during early mitosis and chromosome attractant during late mitosis (PubMed:27362226, PubMed:32879492, PubMed:35513709, PubMed:39153474). Required to maintain individual mitotic chromosomes dispersed in the cytoplasm following nuclear envelope disassembly (PubMed:27362226). During early mitosis, relocalizes from nucleoli to the chromosome surface where it forms extended brush structures that cover a substantial fraction of the chromosome surface (PubMed:27362226). The MKI67 brush structure prevents chromosomes from collapsing into a single chromatin mass by forming a steric and electrostatic charge barrier: the protein has a high net electrical charge and acts as a surfactant, dispersing chromosomes and enabling independent chromosome motility (PubMed:27362226). During mitotic anaphase, the MKI67 brush structure collapses and MKI67 switches from a chromosome repellent to a chromosome attractant to promote chromosome

clustering and facilitate the exclusion of large cytoplasmic particles from the future nuclear space (PubMed:32879492, PubMed:39153474). Mechanistically, dephosphorylation during mitotic exit and simultaneous exposure of a conserved basic patch induce the RNA-dependent formation of a liquid-like condensed phase on the chromosome surface, promoting coalescence of neighboring chromosome surfaces and clustering of chromosomes (PubMed:39153474). Binds premature ribosomal RNAs during anaphase; promoting liquid-liquid phase separation (PubMed:28935370, PubMed:39153474). Binds DNA, with a preference for supercoiled DNA and AT-rich DNA (PubMed:10878551). Does not contribute to the internal structure of mitotic chromosomes (By similarity). May play a role in chromatin organization; it is however unclear whether it plays a direct role in chromatin organization or whether it is an indirect consequence of its function in mitotic chromosome (PubMed:24867636).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab209897 is the carrier-free version of ab92742.
This product is not suitable for xenograft experiments. For further information please contact our Customer Services team.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Ki67 is a nuclear protein often referred to as MKI67 with a molecular weight of approximately 345 kDa. This protein is strongly associated with cell proliferation. Scientists commonly use Ki67 staining techniques to identify and quantify proliferating cells in tissues. Ki67 is expressed in the nucleus during active cell cycle phases—G1 S G2 and M—but not in resting cells in G0 phase. It is detectable through methods such as Ki67 immunofluorescence and Ki67 flow cytometry which are important for analyzing cell division.
Biological function summary	The Ki67 protein plays an essential role in cellular proliferation processes. It does not form part of a stable complex but interacts transiently with other cell cycle-related proteins. Research indicates that Ki67 maintains the structure of the perichromosomal layer during mitosis impacting chromosome separation. It is particularly active in tissues with high cell turnover such as bone marrow and lymphoid organs.
Pathways	Ki67 influences several key cellular pathways that control cell proliferation and differentiation. The protein acts within the regulation of the cell cycle and the PI3K/AKT signaling pathway important for cell growth and survival. Within these pathways Ki67 interacts with proteins like cyclin-dependent kinases (CDKs) which regulate transitions between different phases of the cell cycle.
Associated diseases and disorders	Ki67 has significant implications in oncology and can be used as a biomarker for cancer prognosis. Its expression levels help determine the aggressiveness of tumors especially in breast cancer and prostate cancer. High Ki67 levels correlate with poor prognosis as it indicates rapid cell division. In cancer Ki67 associates with p53 a protein that regulates the cell cycle and function as a tumor suppressor.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

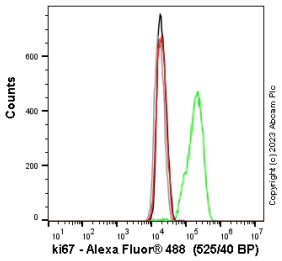
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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22 product images



Flow Cytometry (Intracellular) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Flow Cytometry (Intracellular) staining using rabbit Anti-Ki67 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

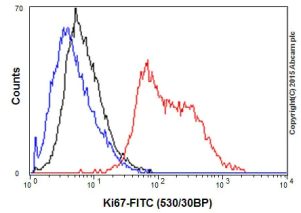
Flow cytometry overlay histogram showing wild-type Hap1 (green line) and MKI67 knockout Hap1 stained with ab92742 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab92742) (1x 106 in 100µl at 0.04 µg/ml (1/57000)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Hap1 - black line, MKI67 knockout Hap1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in Hap1 Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Flow Cytometry (Intracellular) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Flow Cytometry (Intracellular) staining using rabbit Anti-Ki67 antibody

Intracellular Flow Cytometry analysis of Ramos (Human Burkitt's lymphoma cell line) cells lablling Ki67 with purified ab92742 at 1/150 (red). Cells were fixed with 2% paraformaldehyde. An FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype

control, rabbit monoclonal IgG. Blue - Unlabeled control, cells without incubation with primary and secondary antibodies.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

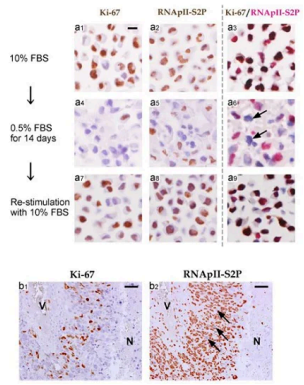


Image from Ishii et al PLoS One. 2016 Jan 22;11(1):e0147366. doi: 10.1371/journal.pone.0147366. eCollection 2016. Fig S4.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Comparison between RNApII-S2P-/low cells and Ki-67- cells
a: Regulation of Ki-67 and RNApII-S2P during proliferation and quiescence in T98G glioblastoma cells. T98G cells were grown in culture medium containing 10% (v/v) fetal bovine serum (FBS), were induced to become quiescent by serum starvation in medium supplemented with 0.5% (v/v) FBS for 14 days, and then were re-stimulated by being split 1:5 into new medium containing 10% (v/v) FBS and cultured for 3 days. The cells were detached from dishes with trypsin-EDTA solution, fixed in 10% (v/v) neutral buffered formalin, and centrifuged. Paraffin sections of the pellet were cut, and expression of Ki-67 and RNApII-S2P was examined by single (brown; a1, a2, a4, a5, a7, a8) or double immunostaining (Ki-67, brown; RNApII-S2P, red; a3, a6, a9). Hematoxylin (blue) was used as a nuclear stain. Ki-67- RNApII-S2P-/low cells (blue cells in the double stained sections) emerged only in the quiescent condition (a6, arrows). Scale bar, 10 μm. b: Single-color immunostaining for Ki-67 (b1) and RNApII-S2P (b2) in serial sections of glioblastoma tissue. Ki-67- tumor cells were frequently found, whereas only a few RNApII-S2P-/low cells (arrows) were observed around necrotic area. N, necrotic area; V, blood vessels. Scale bars, 50 μm.

Ki67 detected using ab92742.

(From Figure S2 of Ishii et al)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

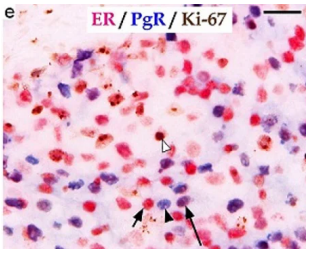


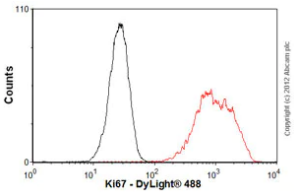
Image from Ishii et al PLoS One. 2016 Jan 22;11(1):e0147366. doi: 10.1371/journal.pone.0147366. eCollection 2016. Fig S4.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Chromogenic triple immunostaining for estrogen receptor (ER), progesterone receptor (PgR), and Ki-67 in breast cancer tissue to verify the triple immunostaining detection method.
Panel e: ER^+ PgR^- $Ki\text{-}67^-$ cells were stained red (short arrow), ER^- PgR^+ $Ki\text{-}67^-$ cells were stained blue (black arrowhead), ER^+ PgR^+ $Ki\text{-}67^-$ cells were stained purple (long arrow), and $Ki\text{-}67^+$ cells were stained brown (white arrowhead). These colors are easily distinguishable. Scale bars, 25 μm.

Deparaffinized sections were pretreated for antigen retrieval by boiling in antigen retrieval solution, pH 9. Sections were incubated with rabbit monoclonal antibody against Ki67 ab92742 at a 1/1000 dilution. After the reaction with (HRP)-conjugated secondary antibodies color was developed with (DAB) and sections were counterstained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

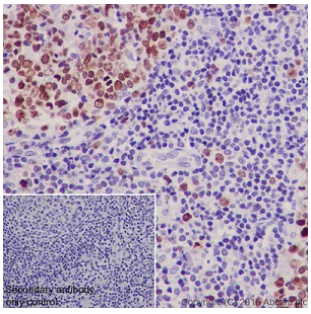


Flow Cytometry (Intracellular) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Flow Cytometry (Intracellular) staining using rabbit Anti-Ki67 antibody

Overlay histogram showing Ramos (Human Burkitt's lymphoma cell line) cells stained with unpurified ab92742 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab92742, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions.
Acquisition of >5,000 events was performed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

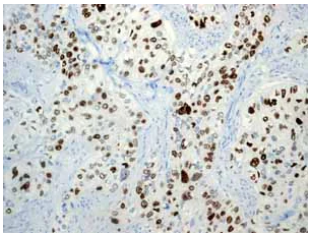
Ki67 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ki67 antibody

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling Ki67 with ab209897 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.
Nucleus staining on human tonsil.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

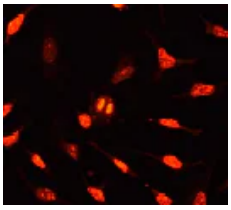


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ki67 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labeling Ki67 with unpurified ab92742.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

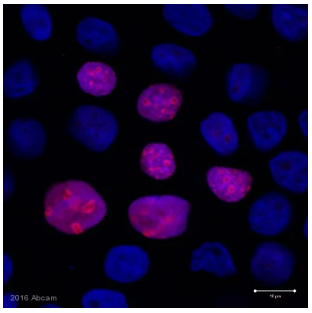
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ki67 antibody

Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Ki67 with unpurified ab92742 at a dilution of 1/250.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).



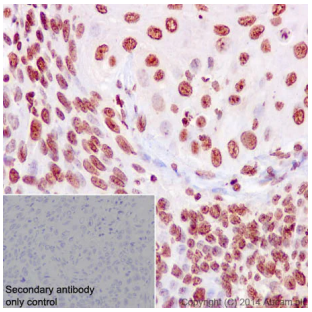
This image is courtesy of an anonymous customer review.

Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ki67 antibody

ab92742 staining Ki67 in human adenocarcinoma cells by ICC/IF (Immunocytochemistry/immunofluorescence).
Cells were fixed with paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS and blocked with 5% serum for 1 hour at 21°C. Samples were incubated with primary antibody (1/1000) for 12 hours at 4°C. A Cy3® conjugated donkey anti-rabbit IgG polyclonal was used as the secondary antibody at a dilution of 1/200.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

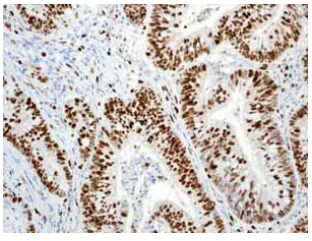


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ki67 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human squamous cell carcinoma of cervix tissue labeling Ki67 with purified ab92742 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Counterstained with hematoxylin.
Negative control using PBS instead of primary antibody (inset).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).



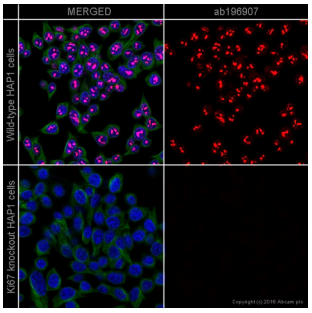
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ki67 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colonic adenocarcinoma tissue labeling Ki67 with unpurified ab92742.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

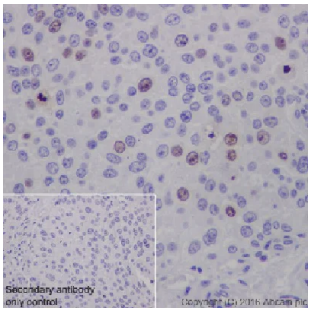


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ki67 antibody

Clone EPR3610 (ab209897) has been successfully conjugated by Abcam. This image was generated using Anti-Ki67 antibody [EPR3610] (Alexa Fluor® 647). Please refer to ab196907 for protocol details.
ab196907 staining Ki67 in wild-type HAP1 cells (top panel) and Ki67 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab196907 at 1/100 dilution (shown in red) and ab195887 at 1/250 dilution (shown in green) overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

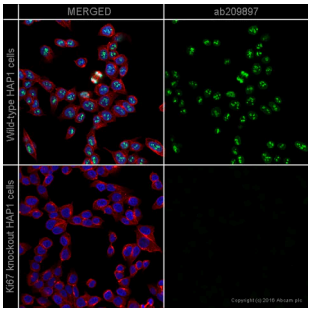
Ki67 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ki67 antibody

Immunohistochemical analysis of paraffin-embedded Human bladder cancer labeling Ki67 with ab209897 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.
Nucleus staining on human bladderr cancer.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution.

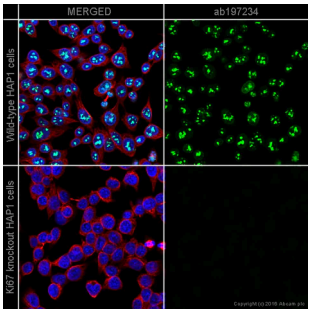
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ki67 antibody

ab209897 staining Ki67 in wild-type HAP1 cells (top panel) and Ki67 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab209897 at 1µg/ml concentration and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

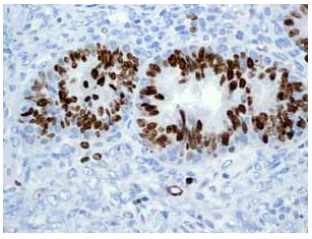


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ki67 antibody

Clone EPR3610 (ab209897) has been successfully conjugated by Abcam. This image was generated using Anti-Ki67 antibody [EPR3610] (Alexa Fluor® 488). Please refer to ab197234 for protocol details.
ab197234 staining Ki67 in wild-type HAP1 cells (top panel) and Ki67 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab197234 at 1/100 dilution (shown in green) and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

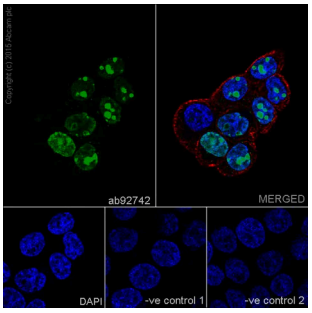


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ki67 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovarian carcinoma tissue labeling Ki67 with unpurified ab92742.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



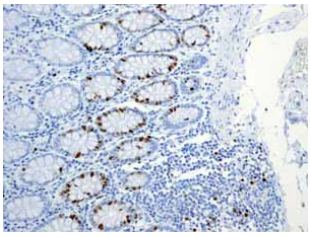
Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ki67 antibody

Immunocytochemistry/Immunofluorescence analysis of HT-29 (Human colorectal adenocarcinoma cell line) cells labeling Ki67 with purified ab92742 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.
Control 1: primary antibody (1/250) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).



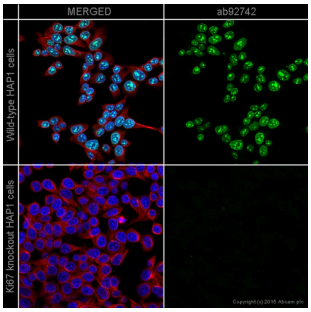
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ki67 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human normal colon tissue labeling Ki67 with unpurified ab92742.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

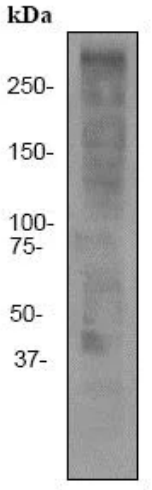
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ki67 antibody

ab92742 staining Ki67 in wild-type HAP1 cells (top panel) and Ki67 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab92742 at 1µg/ml and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labeled in blue with DAPI.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).



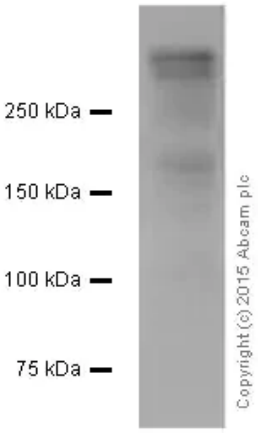
Western blot - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Western blot staining of HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate using rabbit Anti-Ki67 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).
All lanes:
HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

Secondary

All lanes:
HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution



Western blot - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Western blot staining of Ramos (Human Burkitt's lymphoma cell line) cell lysate using rabbit Anti-Ki67 antibody

Blocking and dilution buffer: 5% NFDM/TBST.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

All lanes:
Western blot - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897) at 1/5000 dilution

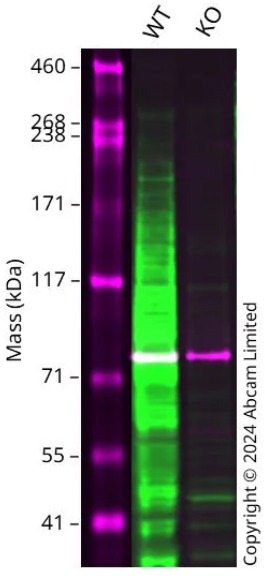
All lanes:
Ramos (Human Burkitt's lymphoma cell line) cell lysate at 20 µg

Secondary

All lanes:
Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 358 kDa

Observed band size: 395 kDa



Western blot - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Ki67 Western blot staining using rabbit Anti-Ki67 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab92742).

Western blot: Anti-MKI67 antibody [EPR3610] (ab92742) staining at 1/5000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab92742 was shown to bind specifically to MKI67. A band was observed at 359 kDa in wild-type A549 cell lysates with no signal observed at this size in MKI67 knockout cell line. To generate this image, wild-type and MKI67 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-Ki67 antibody [EPR3610] (ab92742) at 1/5000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
MKI67 knockout A549 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 359 kDa

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