abcam

ab197547

Anti-Ki67 antibody [SP6] - BSA and Azide free

Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547) is a rabbit recombinant monoclonal antibody provided in a PBS only buffer for easy conjugation detecting Ki67 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF, mIHC**. Suitable for **Human, Mouse, Rat**.

- Clone SP6 is the most cited clone to Ki67
- KO validated for confirmed specificity
- BSA, sodium azide, and glycerol-free for easy conjugation
- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents

Recombinant | RabMAb | Advanced Validation | 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	SP6
Purity	Tissue culture supernatant
Specificity	Ki67 is mainly expressed in proliferating cells. For normal tissue samples (e.g., liver, kidney), no staining may be typically observed due to low level of proliferation and little expression of Ki67. For malignant tissue samples (e.g., colon carcinoma, breast carcinoma), it is more easily to find Ki67 in the proliferating cells of these tissues (PMID: 6206131, 10653597, 34183782). **FURTHER INFORMATION ON SPECIFICITY** **(Chinese Version)**

Epitope	C-terminus

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	If fixing cells in 4% PFA (10 min, room temp), it is recommended to permeabilized cells with 0.1% Triton-X for 5 min.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Common marmoset
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat

Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Common marmoset
Dilution info	-
Notes	-

mIHC

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Common marmoset
Dilution info	-
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/100
Notes	Antigen retrieval: heat mediated antigen retrieval with sodium citrate buffer (pH 6.0)

	Primary antibody condition: primary antibody incubation overnight at +4°C is recommended.

Species	Rat
Dilution info	1/100
Notes	Antigen retrieval: heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) Primary antibody condition: primary antibody incubation overnight at +4°C is recommended.

Species	Human
Dilution info	1/100
Notes	Antigen retrieval: heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) Primary antibody condition: primary antibody incubation overnight at +4°C is recommended.

Predicted

Species	Common marmoset
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Common marmoset
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

What is this antibody validated in?
Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), Multiplex IHC (mIHC) in Human, Mouse, Rat samples.

What is the molecular weight of Ki67?
Anti-Ki67 [SP6] - BSA and Azide free (ab197547) specifically detects a band for Ki67 (UniProt: P46013) at a molecular weight of 358kDa.

Collaboration
Anti-Ki67 [SP6] - BSA and Azide free (ab197547) is a clone from the portfolio of Spring Bioscience (Roche) SP clones which have been optimised for immunohistochemistry (IHC).

Specificity confirmed
The specificity of Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547) has been confirmed by Immunocytochemistry/ Immunofluorescence testing in MKI67 Knockout HAP1 cells.

Other related products
We have a range of other formats of antibody clone [SP6] also available for your convenience: ab16667, ab21700, Carrier free - ab197547, ab231172, ab238624, Oligonucleotide - ab279361, Alexa Fluor® 488 - ab281847, Alexa Fluor® 647 - ab281928, PE - ab282173, APC - ab314285, ab320709, Carrier free - ab320710, ab323292

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

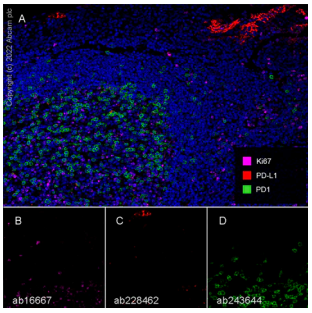
We do not recommend this combination. It is not covered by our product promise.

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.

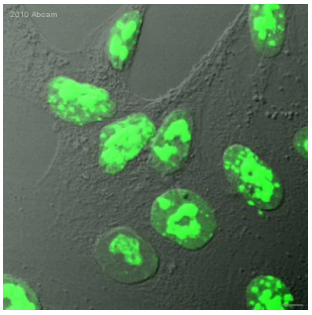
16 product images



Multiplex immunohistochemistry - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

Ki67 Multiplex immunohistochemistry staining using rabbit Anti-Ki67 antibody

This data was developed using ab16667, the same antibody clone in a different buffer formulation. Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil labelling PD1 with ab243644 at 1/500 dilution (1.02 µg/mL) (D), Ki67 with ab16667 at 1/200 dilution (0.15 µg/ml) (B) and PD-L1 with ab228462 at 1/100 dilution (0.52 µg/ml) (C). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. Panel A: merged staining of anti-Ki67 (magenta; Opal™690), anti-PD-L1 (red; Opal™570) and anti-PD1 (green; Opal™520) on human tonsil. Panel B: anti-Ki67 stained on nucleus of proliferating cells. Panel C: anti-PD-L1 stained on membrane of cells involved in T cell inhibition. Panel D: anti-PD1 stained on antigen-stimulated T cells. The section was incubated in three rounds of staining: in the order of ab16667 for 10 mins, ab243644 for 30 mins and ab228462 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

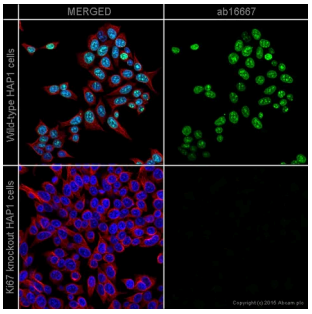


This image is courtesy of a customer review submitted by Peter Zentis

Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

Ki67 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ki67 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab16667). ab16667 staining Ki67 - Proliferation Marker in human HEp-2 cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with Triton X-100 0.25% in PBS. Samples were incubated with primary antibody (1/50 in DPBS) for 1 hour at 21°C. An Atto488-conjugated Donkey anti-rabbit polyclonal (1/50) was used as the secondary antibody. This image was generated from the hybridoma version.



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

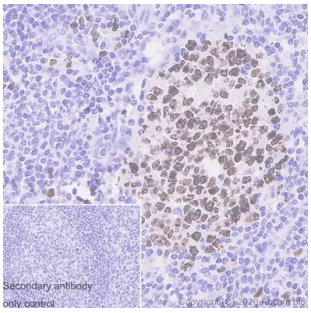
Ki67 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ki67 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab16667).

ab16667 staining Ki67 in wild-type HAP1 cells (top panel) and Ki67 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol for 5 minutes, 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 1 hour. The cells were then incubated with ab16667 at 1/250 dilution and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1 hour with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed

(ab150081) secondary antibody at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

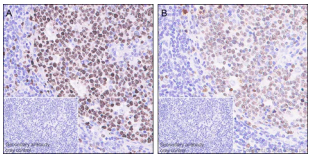
This image was generated from the hybridoma version.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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

This data was developed using the same antibody clone in a different buffer formulation (ab16667).
IHC image of ab16667 staining Ki67 in a section of formalin-fixed paraffin-embedded Human tonsil tissue. The section was pre-treated using heat mediated antigen retrieval with ab93678 (citrate buffer, pH 6.0). The section was then incubated with ab16667, 1/200 (0.156 µg/ml) dilution and detected using ready to use Goat Anti-Rabbit IgG H&L (HRP) antibody. Nuclear staining on human tonsil is observed. The section was incubated with ab16667 overnight at +4°C. DAB was used as the chromogen. The section was then counterstained with haematoxylin.

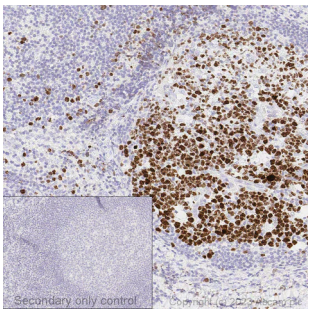


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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

This data was developed using the same antibody clone in a different buffer formulation (ab16667).
Immunohistochemical analysis of formalin-fixed paraffin-embedded human tonsil sections labeling Ki67 with ab16667 at 1/200 dilution (0.156 µg/mL).
Image A:
Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer)
Human tonsil tissue incubated with ab16667 overnight at +4°C.
Heat mediated antigen retrieval using ab93678 (citrate buffer pH 6.0).
Nuclear staining on human tonsil. The section was incubated with ab16667 overnight at +4°C.

Image B:
Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection)
Human tonsil tissue incubated with ab16667 on a Leica Biosystems BOND® RX instrument.
Heat mediated antigen retrieval with Citrate buffer (pH 6.0 epitope retrieval solution2) for 20 mins.

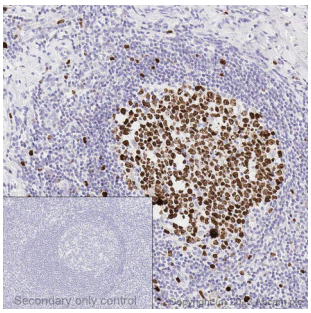


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil Ki67 with ab197547 at a concentration of 5µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with ULTRA cell conditioning solution (CC1 pH8.5). ab197547 anti Ki67 antibody was incubated at 37oC for 16min. Sections were

counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

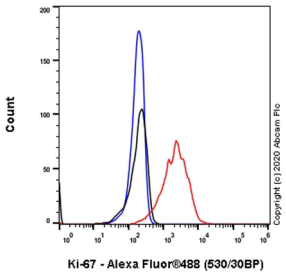


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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

This data was developed using the same antibody clone in a different buffer formulation (ab16667).

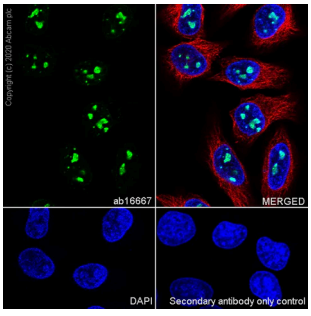
Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling Ki67 with ab16667 at 1/500 dilution. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with ULTRA cell conditioning solution (CC1 pH8.5). ab16667 anti Ki67 antibody was incubated at 37°C for 16min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

Ki67 Flow Cytometry (Intracellular) staining of HAP1 cells using rabbit Anti-Ki67 antibody

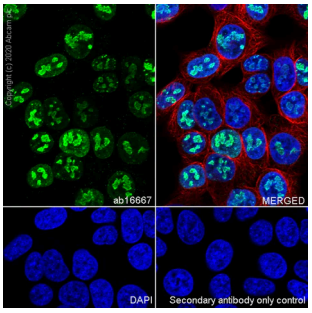
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized parental HAP1 (Wildtype controlHuman chronic myelogenous leukemianear-haploid cell line) cells labelling Ki67 with ab16667 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730)(Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488 ab150077) at 1/2000 dilution was used as the secondary antibody.
This data was developed using ab16667, the same antibody clone in a different buffer formulation.



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

Ki67 Immunocytochemistry/ Immunofluorescence staining of HeLa cells using rabbit Anti-Ki67 antibody

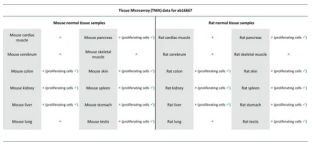
This data was developed using ab16667, the same antibody clone in a different buffer formulation. Immunofluorescent analysis of 100% methanol-fixed None permeabilized HeLa cells labelling Ki67 with ab16667 at 1/1000 dilution followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (2 µg/mL) (Green). Confocal image showing nucleolar staining in HeLa cell line ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 µg/mL) (Red). The Nuclear counterstain was DAPI (Blue).Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1000 dilution (2 µg/mL).



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

Ki67 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ki67 antibody

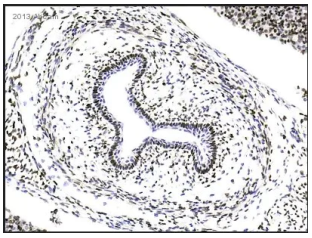
This data was developed using ab16667, the same antibody clone in a different buffer formulation.Immunofluorescent analysis of 100% methanol-fixed, None permeabilized parental HAP1 cells labelling Ki67 with ab16667 at 1/1000 dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (2 µg/mL) (Green). Confocal image showing nucleolar staining in parental HAP1cell line ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 µg/mL) (Red). The Nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1000 dilution (2 µg/mL).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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

Tissue Microarrays stained for Ki67 using ab16667 at 1/200 dilution (0.145 µg/ml) in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using ab93678 (citrate buffer, pH 6.0). The sections were incubated with ab16667 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer) (ab214880). Hematoxylin was used as the counterstain.
This data was developed using the same antibody clone in a different buffer formulation (ab16667).



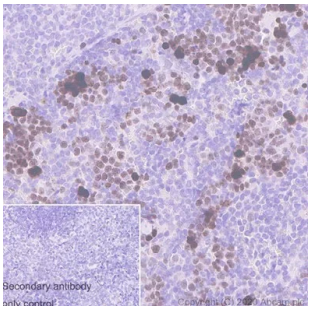
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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

This data was developed using the same antibody clone in a different buffer formulation (ab16667).

ab16667 staining Ki67 in rat oesophagus by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue was fixed with formaldehyde and a heat mediated antigen retrieval step was performed using citrate buffer. Samples were then blocked with 1% BSA for 10 minutes at 21°C followed by incubation with the primary antibody for 30 minutes at 1/100. A biotin-conjugated goat anti-rabbit polyclonal was used as secondary antibody at a 1/250 dilution.

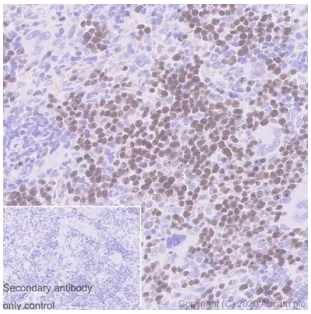
This image was generated from the hybridoma version.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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

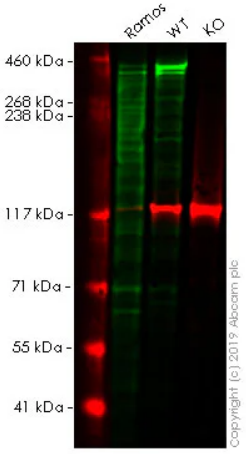
This data was developed using the same antibody clone in a different buffer formulation (ab16667).
IHC image of ab16667 staining Ki67 in a section of formalin-fixed paraffin-embedded Mouse spleen tissue. The section was pre-treated using heat mediated antigen retrieval with ab93678 (citrate buffer, pH 6.0). The section was then incubated with ab16667, 1/200 (0.156 µg/mL) dilution and detected using a ready to use Goat Anti-Rabbit IgG H&L (HRP) antibody. Nuclear staining on mouse spleen.The section was incubated with ab16667 overnight at +4°C. DAB was used as the chromogen. The section was then counterstained with haematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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

This data was developed using the same antibody clone in a different buffer formulation (ab16667).
IHC image of ab16667 staining Ki67 in a section of formalin-fixed paraffin-embedded Rat spleen tissue. The section was pre-treated using heat mediated antigen retrieval with (citrate buffer, pH 6.0). The section was then incubated with ab16667, 1/200 (0.156 µg/mL) dilution and detected using ready to use Goat Anti-Rabbit IgG H&L (HRP) antibody. Nuclear staining on rat spleen. The section was incubated with ab16667 overnight at +4°C. DAB was used as the chromogen. The section was then counterstained with haematoxylin.



Western blot - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

Ki67 Western blot staining using rabbit Anti-Ki67 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab16667).
Lanes 1-3: Merged signal (red and green). Green - ab16667 observed at 359 kDa. Red - loading control, ab130007 observed at 125 kDa.

ab16667 was shown to react with Ki67 in wild-type HeLa. Loss of signal was observed when knockout sample ab263762 was used. Wild-type and Ki67 knockout samples were subjected to SDS-PAGE. ab16667 and Anti-Vinculin antibody VIN-54] (ab130007) were incubated overnight at 4°C at 1 in 100 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Ki67 antibody [SP6] (ab16667) at 1/100 dilution

Lane 1:
Ramos cell lysate at 20 µg

Lane 2:

Wild-type HeLa cell lysate at 20 µg

Lane 3:
Western blot - Human MKI67 (Ki67) knockout HeLa cell lysate (ab263762) at 20 µg

Secondary

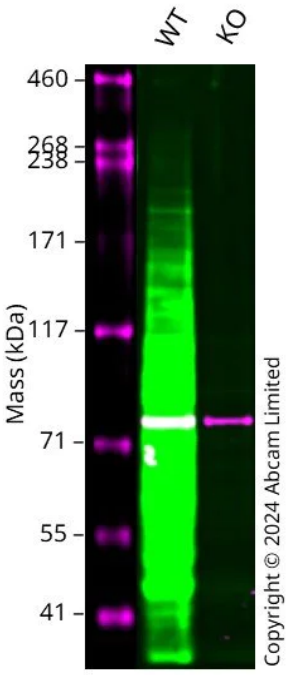
All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 358 kDa

Observed band size: 124 kDa, 359 kDa

Western blot - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)



Ki67 Western blot staining using rabbit Anti-Ki67 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab16667).

Western blot: Anti-MKI67 antibody [SP6] (ab16667) staining at 1/500 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab16667 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 [SP6] (ab16667) at 1/500 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

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.