abcam

Product datasheet

Anti-Ki67 antibody [SP6] - BSA and Azide free ab197547

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 2 References 17 Images

Overview

Product name	Anti-Ki67 antibody [SP6] - BSA and Azide free
Description	Rabbit monoclonal [SP6] to Ki67 - BSA and Azide free
Host species	Rabbit
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)**
Tested applications	**Suitable for: WB, mIHC, Flow Cyt (Intra), IHC-P, ICC/IF**
Species reactivity	**Reacts with: Mouse, Rat, Human** **Predicted to work with: Common marmoset** ⚠
Immunogen	**Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.**
Epitope	**C-terminus**
Positive control	**WB: HeLa cell lysate. IHC-P: Human tonsil and testis tissue. Common marmoset spleen tissue. Rat esophagus, small intestine and liver tissue. Mouse embryonic skin tissue. IHC-Fr: Rat lymph node tissue, Transgenic mouse spinal cord tissue. ICC/IF: HeLa and HAP1 cells. Human cardiac stem cells. HEp-2 cells. Rat cardiomyocytes. Flow Cyt (intra): HAP1 cells. mIHC: Human tonsil**
General notes	**ab197547 is the carrier-free version of ab16667**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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.

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 production

For more information **see here**.

This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	SP6
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab197547 in the following tested applications.

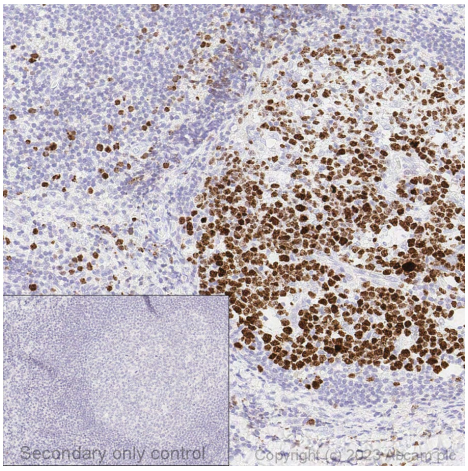
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 358 kDa.
mIHC		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IHC-P	★★★★★ **(1)**	1/100. 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.
ICC/IF		Use at an assay dependent concentration. If fixing cells in 4% PFA (20 min, room temp), it is recommended to permeabilized cells with 0.1% Triton-X for 5 min.

Target

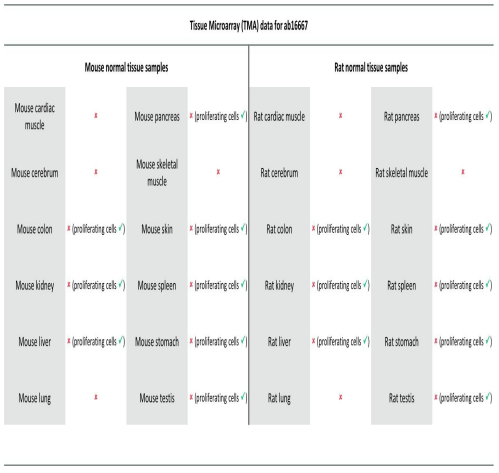
Function	Required to maintain individual mitotic chromosomes dispersed in the cytoplasm following nuclear envelope disassembly (PubMed:27362226). Associates with the surface of the mitotic chromosome, the perichromosomal layer, and covers a substantial fraction of the chromosome surface (PubMed:27362226). 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). 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 (PubMed:24867636). It is however unclear whether it plays a direct role in chromatin organization or whether it is an indirect consequence of its function in maintaining mitotic chromosomes dispersed.
Sequence similarities	Contains 1 FHA domain. Contains 16 K167R repeats. Contains 1 PP1-binding domain.
Developmental stage	Expression occurs preferentially during late G1, S, G2 and M phases of the cell cycle, while in cells in G0 phase the antigen cannot be detected (at protein level) (PubMed:6206131). Present at highest level in G2 phase and during mitosis (at protein level). In interphase, forms fiber-like structures in fibrillarin-deficient regions surrounding nucleoli (PubMed:2674163, PubMed:8799815).
Post-translational modifications	Phosphorylated. Hyperphosphorylated in mitosis (PubMed:10502411, PubMed:10653604). Hyperphosphorylated form does not bind DNA.
Cellular localization	Chromosome. Nucleus. Nucleus, nucleolus. Associates with the surface of the mitotic chromosome, the perichromosomal layer, and covers a substantial fraction of the mitotic chromosome surface (PubMed:27362226). Associates with satellite DNA in G1 phase (PubMed:9510506). Binds tightly to chromatin in interphase, chromatin-binding decreases in mitosis when it associates with the surface of the condensed chromosomes (PubMed:15896774, PubMed:22002106). Predominantly localized in the G1 phase in the perinucleolar region, in the later phases it is also detected throughout the nuclear interior, being predominantly localized in the nuclear matrix (PubMed:22002106).

Images



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

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.

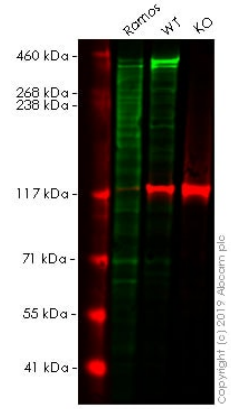


Tissue Microarray (TMA) data for ab16667

Mouse normal tissue samples				Rat normal tissue samples			
Mouse cardiac muscle	x	Mouse pancreas	x (proliferating cells √)	Rat cardiac muscle	x	Rat pancreas	x (proliferating cells √)
Mouse cerebrum	x	Mouse skeletal muscle	x	Rat cerebrum	x	Rat skeletal muscle	x
Mouse colon	x (proliferating cells √)	Mouse skin	x (proliferating cells √)	Rat colon	x (proliferating cells √)	Rat skin	x (proliferating cells √)
Mouse kidney	x (proliferating cells √)	Mouse spleen	x (proliferating cells √)	Rat kidney	x (proliferating cells √)	Rat spleen	x (proliferating cells √)
Mouse liver	x (proliferating cells √)	Mouse stomach	x (proliferating cells √)	Rat liver	x (proliferating cells √)	Rat stomach	x (proliferating cells √)
Mouse lung	x	Mouse testis	x (proliferating cells √)	Rat lung	x	Rat testis	x (proliferating cells √)

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

Tissue Microarrays stained for "Anti-Ki67 antibody [SP6]" 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. Taking mouse spleen tissue as an example, Ki67 is barely expressed or the expression level is very low in normal liver tissue, and the IHC test result is usually negative. While the expression of Ki67 can be upregulated in the proliferating cells of spleen tissue, and the IHC test result could be positive.
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 counter stain.



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

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

Lane 1 : Ramos cell lysate
Lane 2 : Wild-type HeLa cell lysate
Lane 3 : MKI67 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

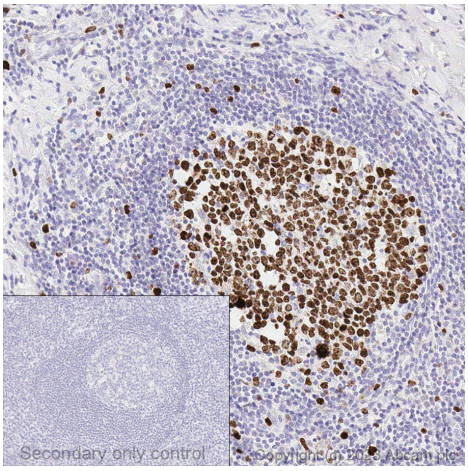
Performed under reducing conditions.

Predicted band size: 358 kDa
Observed band size: 359 kDa

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.

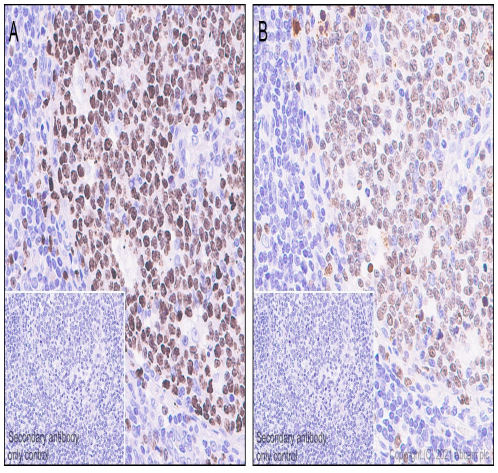
ab16667 was shown to react with Ki67 in wild-type HeLa. Loss of signal was observed when knockout sample **ab263762** was used. Wild-type and Ki67 knockout samples were subjected to SDS-PAGE. **ab16667** and Anti-Vinculin antibody VIN-54] (**ab130007**) were incubated overnight at 4°C at 1 in 100 dilution 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.



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

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.



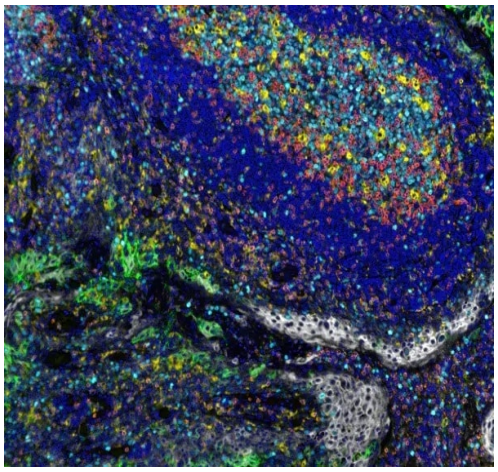
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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 (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.



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

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

Fluorescence multiplex immunohistochemical analysis of normal human tonsil tissue (formalin-fixed paraffin-embedded section).

Merged staining of anti-PD1 (**ab237728**; orange; Opal™520), anti-PDL1 (**ab237726**; green; Opal™540), anti-CD68 (**ab192847**; yellow; Opal™570), anti-CD3 (**ab16669**; red; Opal™620), anti-Ki67 (**ab16667**; light blue; Opal™650) and anti-PanCK (**ab7753**; grey; Opal™690).

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 7-color automation IHC kit (NEL821001KT, Akoya Biosciences®).

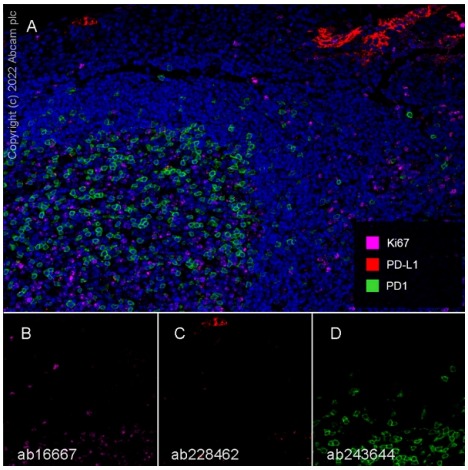
The section was incubated in six rounds of staining; in the order of **ab237728** (1/500 dilution), **ab237726** (1/500 dilution), **ab192847** (1/300 dilution), **ab16669** (1/300 dilution), **ab16667** (1/200 dilution) and **ab7753** (1/200 dilution); each using a separate fluorescent tyramide signal amplification system.

Sodium citrate antigen retrieval (Leica ER1, pH6.0, 30 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.

DAPI (dark blue) was used as a nuclear counter stain.

Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra Polaris.

This image was generated from the hybridoma version.



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

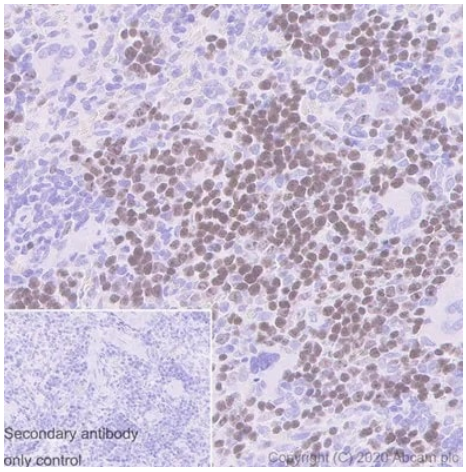
This data was developed using **ab16667**, the same antibody clone in a different buffer formulation.

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.

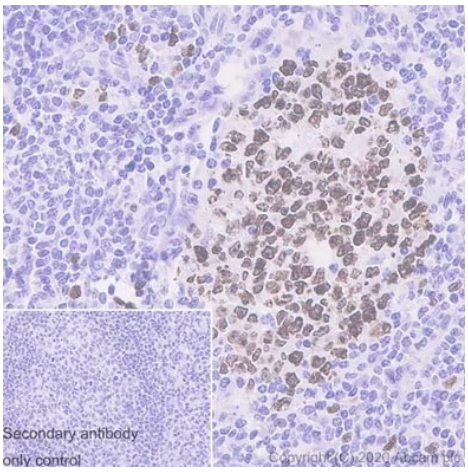
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



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

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

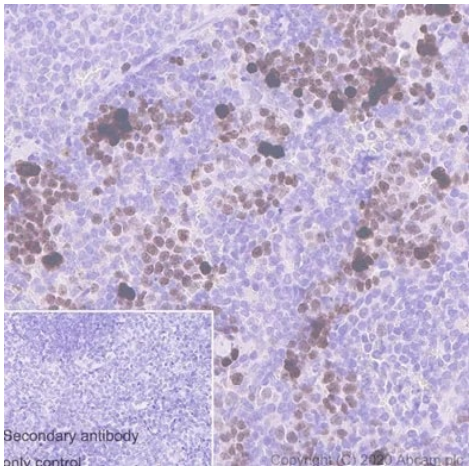
IHC image of **ab16667** staining Ki67 in a section of formalin-fixed paraffin-embedded Rat spleen tissue. The section was pre-treated using heat mediated antigen retrieval with (citrate buffer, pH 6.0). The section was then incubated with **ab16667**, 1/200 (0.156 µg/mL) dilution and detected using 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.



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

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

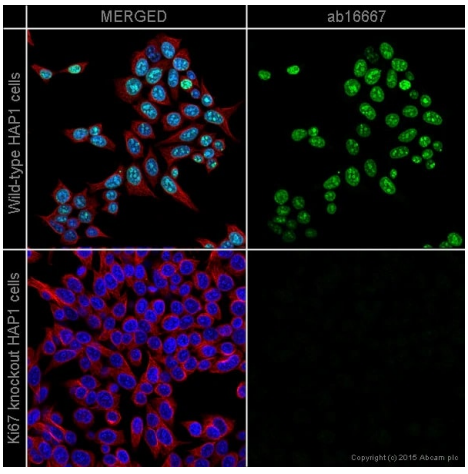
IHC image of **ab16667** staining Ki67 in a section of formalin-fixed paraffin-embedded Human tonsil tissue. The section was pre-treated using heat mediated antigen retrieval with **ab93678** (citrate buffer, pH 6.0). The section was then incubated with **ab16667**, 1/200 (0.156 µg/ml) dilution and detected 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)

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.

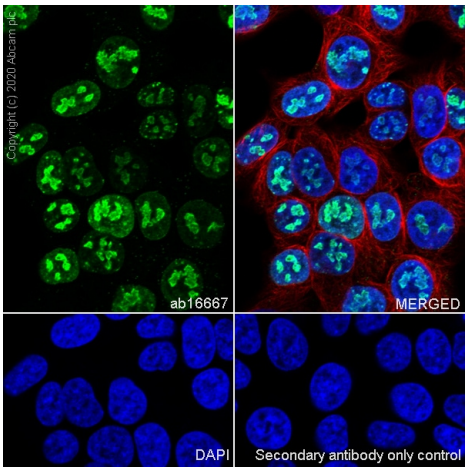


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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.

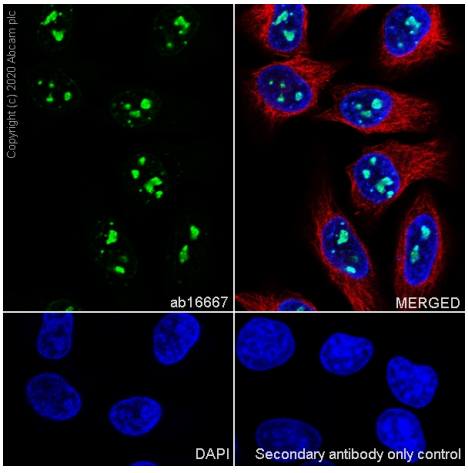


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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).

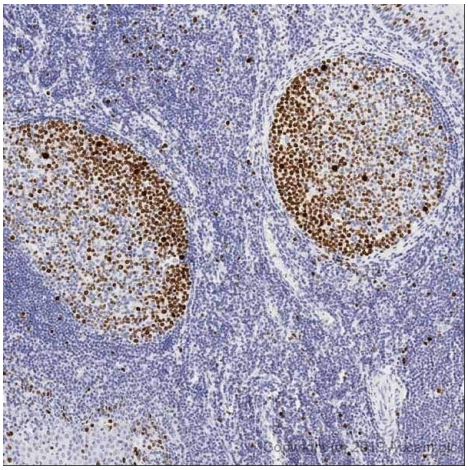


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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).

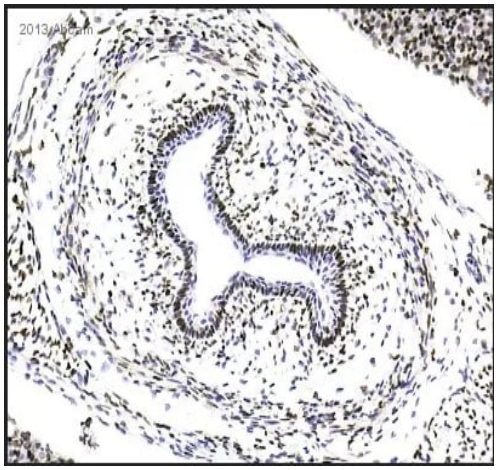


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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 normal human tonsil* performed on a Leica BONDTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20mins. The section was then incubated with **ab16667**, 1/200 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

This image was generated from the hybridoma version.

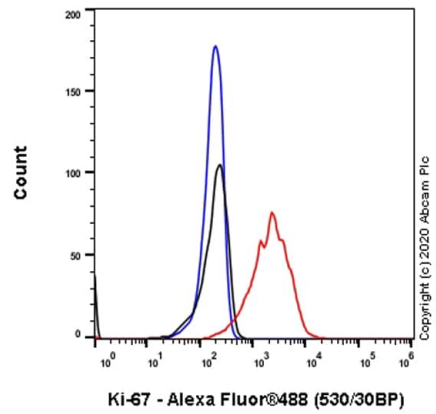


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] - BSA and Azide free (ab197547)

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 21ºC 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.

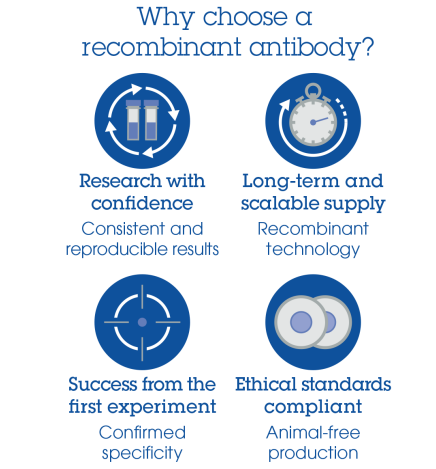
This image was generated from the hybridoma version.



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

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

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized parental HAP1 (Wildtype control Human chronic myelogenous leukemia near-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.



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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

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