

Anti-Ki67 antibody [SP6] ab16667


KO 評価済 リコンビナント RabMAb

★★★★★ [126 Abreviews](#) [2272 References](#) [画像数 26](#)

製品の概要

製品名	Anti-Ki67 antibody [SP6]
製品の詳細	Rabbit monoclonal [SP6] to Ki67
由来種	Rabbit
特異性	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)

アプリケーション	<u>適用あり: Flow Cyt (Intra), IHC-P, WB, mlHC, ICC/IF</u>
種交差性	<u>交差種: Mouse, Rat, Human</u> <u>交差が予測される動物種: Common marmoset</u> 
免疫原	<u>Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.</u>
エピトープ	<u>C-terminus</u>
ポジティブ・コントロール	<u>WB: HeLa cell lysate. IHC-P: Human tonsil, rat and mouse spleen tissues. Human colon carcinoma. ICC/IF: HeLa and HAP1 cells. Flow Cyt (intra): HAP1 cells. mlHC: Human tonsil</u>

特記事項	<u>ab16667 was switched from a hybridoma to recombinant production method on 24th October 2019.</u> <u>This product is a recombinant monoclonal antibody, which offers several advantages including:</u> <u>- High batch-to-batch consistency and reproducibility</u> <u>- Improved sensitivity and specificity</u> <u>- Long-term security of supply</u> <u>- Animal-free production</u> <u>For more information see here.</u>
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Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to [RabMAb[®] patents](#).

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

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.20 Preservative: 0.1% Sodium azide Constituents: 1% BSA, PBS
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	SP6
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab16667の使用に適用されます**
 アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/1000. ab172730 , Rabbit monoclonal isotype, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★★ (63)	1/200. 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.
WB	★★★★★ (3)	Use at an assay dependent concentration. Predicted molecular weight: 358 kDa.
mlHC		1/200.
ICC/IF	★★★★★ (22)	1/250. If fixing cells in 4% PFA (20 min, room temp), it is recommended to permeabilized cells with 0.1% Triton-X for 5 min.

ターゲット情報

機能	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
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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.

配列類似性

Contains 1 FHA domain.
Contains 16 K167R repeats.
Contains 1 PP1-binding domain.

発生段階

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).

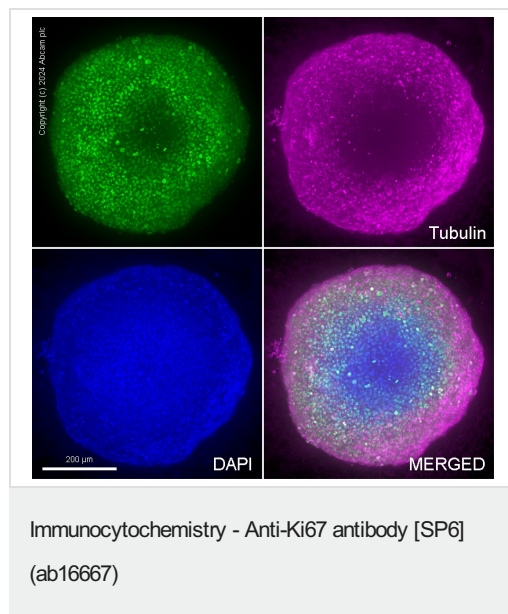
翻訳後修飾

Phosphorylated. Hyperphosphorylated in mitosis (PubMed:10502411, PubMed:10653604). Hyperphosphorylated form does not bind DNA.

細胞内局在

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).

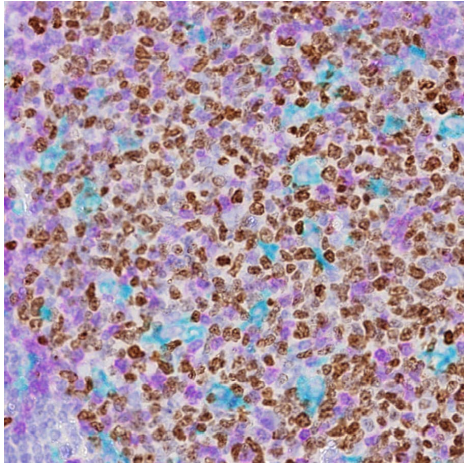
画像



ab16667 staining of Ki67 in a HCT116 cell spheroid. The cells were fixed with 100% methanol (5 min), permeabilised with 0.5% Triton X-100 for 1h and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween overnight at room temperature. The spheroids were then incubated overnight at room temperature with ab16667 at 2 µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin at 2 µg/ml. DAPI was used as nuclear counterstain (shown in blue). As secondary antibodies **ab150081** Goat anti-Rabbit (Alexa Fluor® 488) (shown in green) and **ab150120** Goat anti-Mouse (Alexa Fluor® 594) (shown in magenta) were used, incubated overnight at room temperature. All permeabilization, blocking and antibody incubation steps were performed using a rotary shaker.

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

The antibody ab16667 also worked using 4% formaldehyde fixation (10 min).



Multiplex immunohistochemistry - Anti-Ki67 antibody [SP6] (ab16667)

Chromogenic multiplex immunohistochemical staining of FFPE normal human tonsil tissue. Ab16667, anti-Ki67 DAB chromogen. Ab16669, anti-CD3 purple chromogen and **ab192847**, anti-CD68 teal chromogen plus haematoxylin II counterstain.

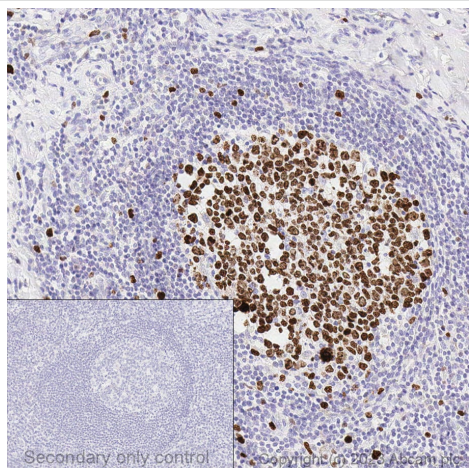
Chromogenic immunostaining was performed on a Roche Ventana Benchmark Ultra instrument. The section was deparaffinised and incubated with CC1 solution for 24min, 100°C. Following this, with 3 rounds of staining in the order of ab16667 (1/500), **ab192847** (1/4000) **ab16669** (1/1000). Between rounds of staining, antibody denaturation was conducted using Ultra CC2 solution for 8min at 100°C to avoid cross reactivity. Signal was developed with anti-rabbit HQ followed by anti-HQ HRP coupled with Chromomap DAB kit, Discovery purple or Discovery teal chromogens and haematoxylin II counterstain.

Tissue Microarray (TMA) data for ab16667							
Mouse normal tissue samples				Rat normal tissue samples			
Mouse cardiac muscle	+	Mouse pancreas	+	Rat cardiac muscle	+	Rat pancreas	+
Mouse cerebrum	+	Mouse skeletal muscle	+	Rat cerebrum	+	Rat skeletal muscle	+
Mouse colon	+	Mouse skin	+	Rat colon	+	Rat skin	+
Mouse kidney	+	Mouse spleen	+	Rat kidney	+	Rat spleen	+
Mouse liver	+	Mouse stomach	+	Rat liver	+	Rat stomach	+
Mouse lung	+	Mouse testis	+	Rat lung	+	Rat testis	+

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] (ab16667)

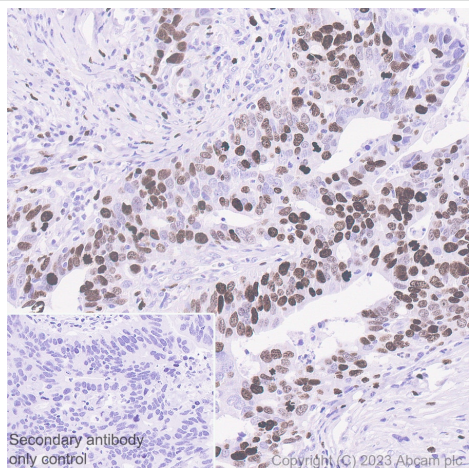
Tissue Microarrays stained for "Anti-Ki67 antibody [SP6]" using "ab16667" at 1/200 dilution (0.145 µg/ml) in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. Taking mouse spleen tissue as an example, 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.



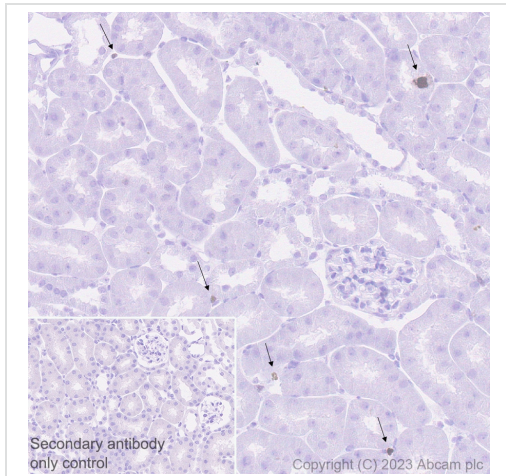
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] (ab16667)

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 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] (ab16667)

IHC image of ab16667 staining Ki67 in a section of formalin-fixed paraffin-embedded Human colon carcinoma tissue. The section incubated with ab16667 at 1/200 (0.145 µg/ml) dilution and ready to use Goat Anti-Rabbit IgG H&L (HRP polymer) ([ab214880](#)) was used as secondary antibody. Positive staining on human colon carcinoma. The section was incubated with ab16667 at 4°C overnight. The section was counterstained with haematoxylin. Heat mediated antigen retrieval was performed using [ab93678](#) (citrate buffer, pH 6.0).

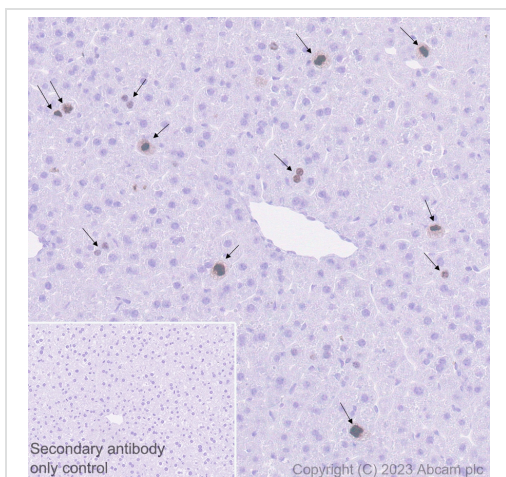


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] (ab16667)

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling Ki67 with ab16667 at 1/200 (0.145 µg/ml) followed by a ready to use LGoat Anti-Rabbit IgG H&L (HRP polymer) secondary antibody ([ab214880](#)). **Low expression:** positive staining only on proliferating cells (arrow) of mouse kidney. The section was incubated with ab16667 at 4°C overnight and counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer) ([ab214880](#)).

Heat mediated antigen retrieval was performed using [ab93678](#) (citrate buffer, pH 6.0).

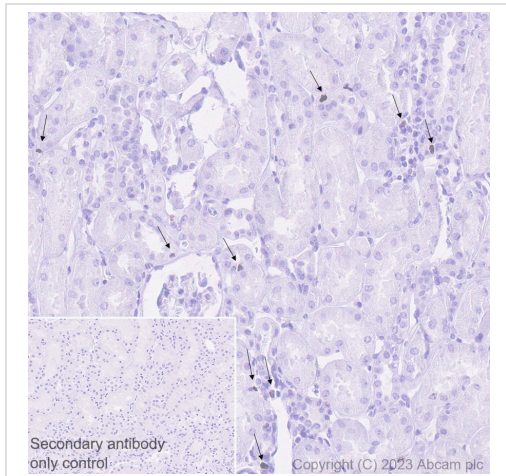


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] (ab16667)

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling Ki67 with ab16667 at 1/200 (0.145 µg/ml) followed by a ready to use LGoat Anti-Rabbit IgG H&L (HRP polymer) secondary antibody ([ab214880](#)). **Low expression:** positive staining only on proliferating cells (arrow) of mouse liver. The section was incubated with ab16667 at 4°C overnight and counterstained with Hematoxylin.

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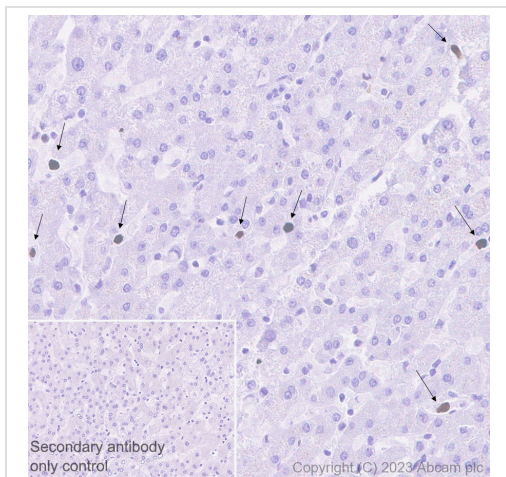


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Tissue Microarray (TMA) data for ab16667

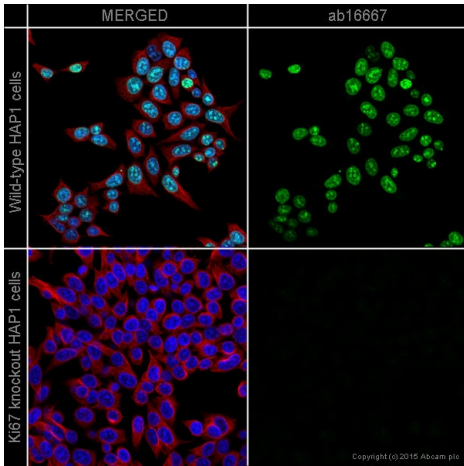
Normal tissue samples			Malignant tissue samples		
Human cardiac muscle	x	Human placenta	Human placenta	proliferating cells +	
Human cerebellum	x	Human skeletal muscle	Human skeletal muscle	proliferating cells +	
Human colon	proliferating cells +	Human skin	Human skin	proliferating cells +	
Human endometrium	x	Human spleen	Human spleen	proliferating cells +	
Human kidney	proliferating cells +	Human stomach	Human stomach	proliferating cells +	
Human liver	proliferating cells +	Human testis	Human testis	proliferating cells +	
Human lung	x	Human thyroid	Human thyroid	proliferating cells +	
Human mammary gland	proliferating cells +	Human tonsil	Human tonsil	proliferating cells +	
Human pancreas	proliferating cells +				
		Clear cell carcinoma of human kidney	Clear cell carcinoma of human kidney	proliferating cells +	
		Human bladder cancer	Human bladder cancer	proliferating cells +	
		Human breast carcinoma	Human breast carcinoma	proliferating cells +	
		Human cervical carcinoma	Human cervical carcinoma	proliferating cells +	
		Human colon carcinoma	Human colon carcinoma	proliferating cells +	
		Human endometrial carcinoma	Human endometrial carcinoma	proliferating cells +	
		Human gastric adenocarcinoma	Human gastric adenocarcinoma	proliferating cells +	
		Human glioma	Human glioma	proliferating cells +	
		Human hepatocellular carcinoma	Human hepatocellular carcinoma	proliferating cells +	
		Human lung carcinoma	Human lung carcinoma	proliferating cells +	
		Human ovarian carcinoma	Human ovarian carcinoma	proliferating cells +	
		Human pancreatic carcinoma	Human pancreatic carcinoma	proliferating cells +	
		Human prostatic hyperplasia	Human prostatic hyperplasia	proliferating cells +	
		Human thyroid carcinoma	Human thyroid carcinoma	proliferating cells +	

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] (ab16667)

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 human colon tissue as an example, Ki67 is barely expressed or the expression level is very low in normal colon tissue, and the IHC test result is usually negative. While the expression of Ki67 can be upregulated in the proliferating cells of colon tissue, and the IHC test result could be positive.

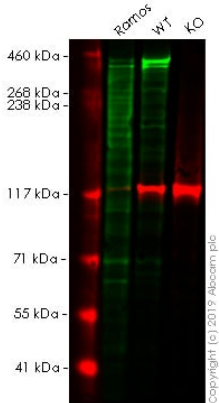
The sections were pre-treated using Heat mediated antigen retrieval using [ab93678](#) (citrate buffer, pH 6.0). The sections were incubated with ab16667 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer) ([ab214880](#)). Hematoxylin was used as the counter stain.



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6] (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.



Western blot - Anti-Ki67 antibody [SP6] (ab16667)

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

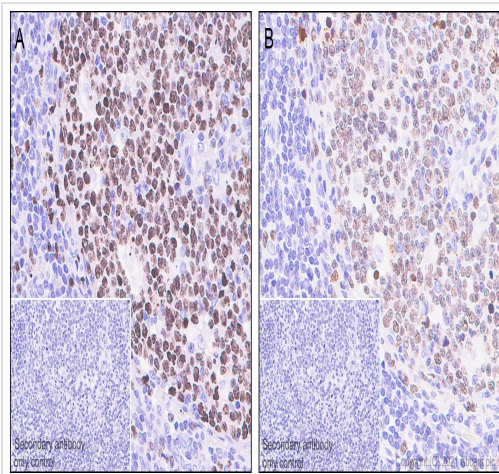
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 358 kDa

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 cells. Loss of signal was observed when knockout cell line **ab255407** (knockout cell lysate **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] (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).

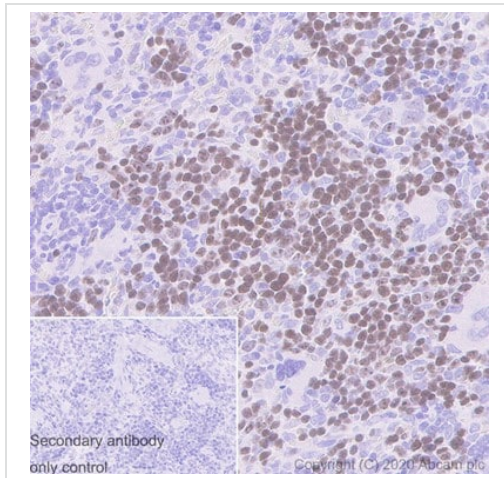
Nuclear staining on human tonsil. The section was incubated with ab16667 overnight at +4°C.

Image B:

Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection)

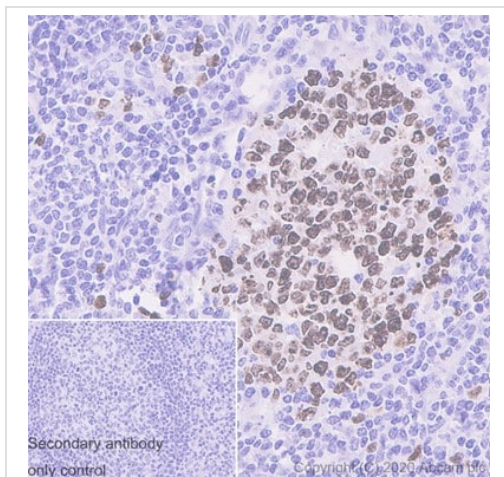
Human tonsil tissue incubated with ab16667 on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution2) for 20 mins.



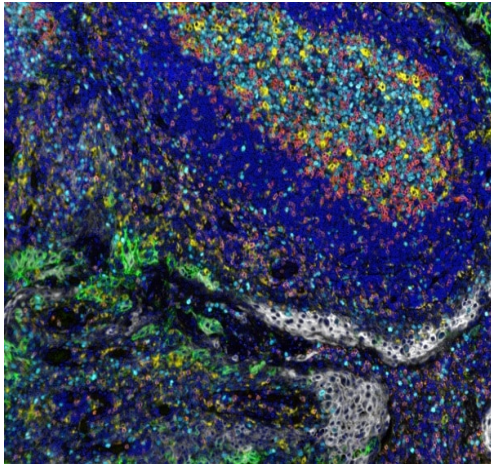
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] (ab16667)

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] (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.



Multiplex immunohistochemistry - Anti-Ki67 antibody [SP6] (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®).

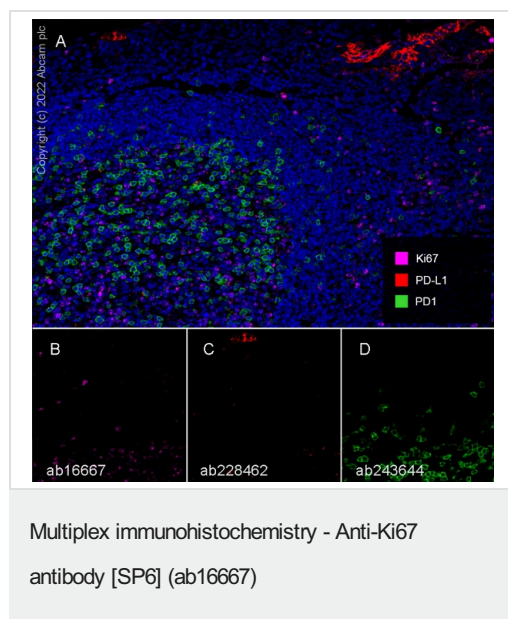
The section was incubated in six rounds of staining; in the order of [ab237728](#) (1/500 dilution), [ab237726](#) (1/500 dilution), [ab192847](#) (1/300 dilution), [ab16669](#) (1/300 dilution), ab16667 (1/200 dilution) and [ab7753](#) (1/200 dilution); each using a separate fluorescent tyramide signal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



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.

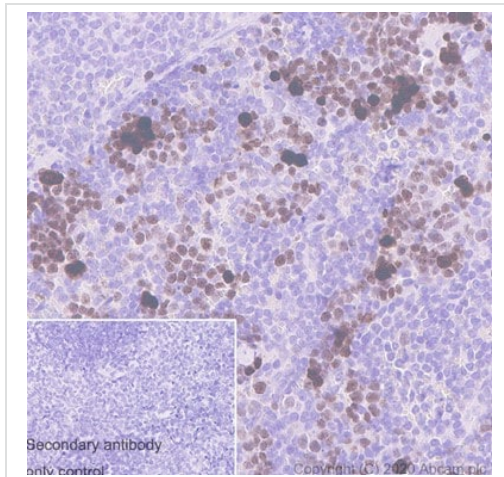
Panel B: anti-Ki67 stained on nucleus of proliferating cells.

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.

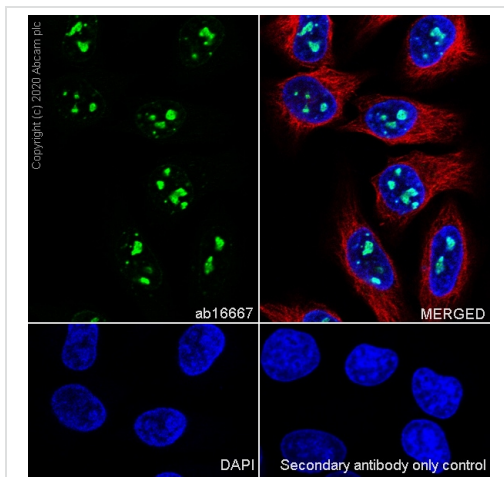
The section was incubated in three rounds of staining: in the order of ab16667 for 10 mins, **ab243644** for 30 mins and **ab228462** for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

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.



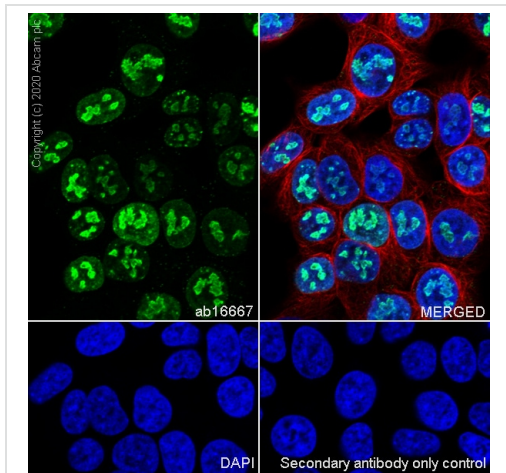
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] (ab16667)

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] (ab16667)

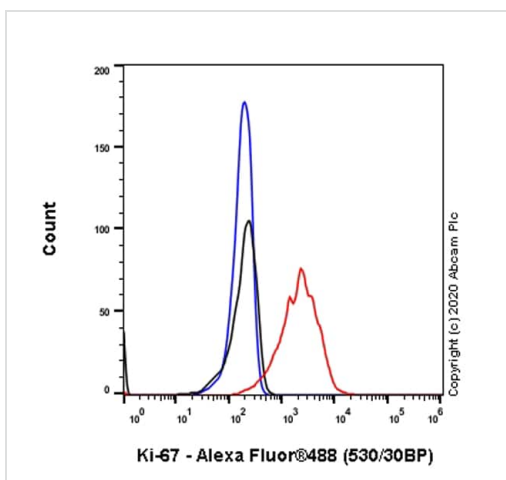
Immunofluorescent analysis of 100% methanol-fixed, permeabilized HeLa cells labelling Ki67 with ab16667 at 1/1000 dilution, followed by [ab150077](#) Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (2 µg/mL) (Green). Confocal image showing nucleolar staining in HeLa cell line [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).



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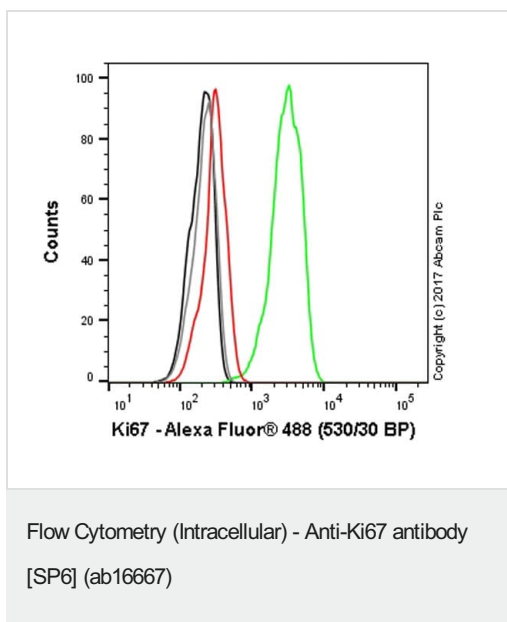
Immunofluorescent analysis of 100% methanol-fixed, None permeabilized parental HAP1 cells labelling Ki67 with ab16667 at 1/1000 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (2 µg/mL) (Green). Confocal image showing nucleolar staining in parental HAP1 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).

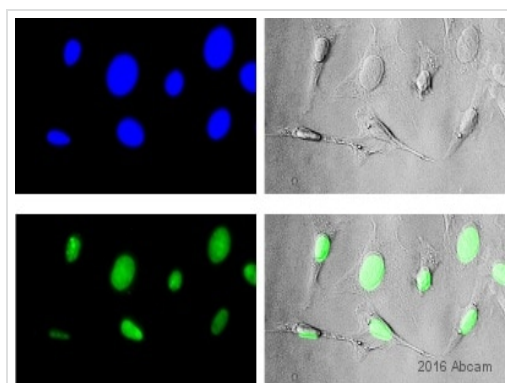


Flow Cytometry (Intracellular) - Anti-Ki67 antibody [SP6] (ab16667)

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.



Overlay histogram showing HAP1 wildtype (green line) and HAP1-MKI67 knockout cells (red line) stained with ab16667. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab16667, 1/1000) for 30 min at 22°C. The secondary antibody used was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150081**) secondary antibody at 1/2000 dilution for 30 min at 22°C. A Rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-MKI67 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This image was generated from the hybridoma version.

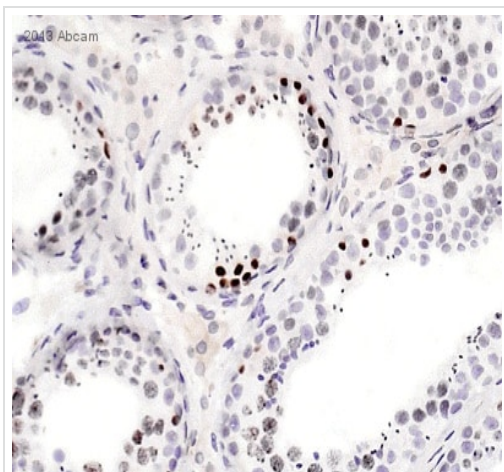


Immunocytochemistry/ Immunofluorescence analysis of human cardiac stem cells labeling Ki67 with ab16667 at 1/250 dilution. Cells were fixed in paraformaldehyde and permeabilized with Triton x-100, 0.01%. Cells were blocked in BSA for 1 hour at room temperature. A polyclonal chicken anti-rabbit Alex Fluor® 488 secondary antibody was used at 1/500 dilution.

This image was generated from the hybridoma version.

Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6] (ab16667)

This image is courtesy of an anonymous abreview.

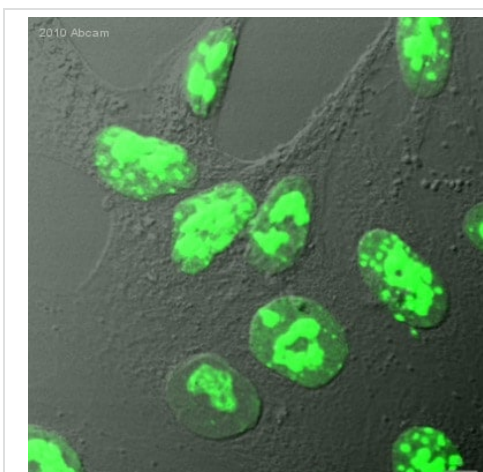


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [SP6] (ab16667)

Image Courtesy of Carl Hobbs, King College London, U.K.

ab16667 staining Ki67 in human testis 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 2 hours 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.



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6] (ab16667)

This image is courtesy of an Abreview submitted by Peter Zentis

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.

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Anti-Ki67 antibody [SP6] (ab16667)

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