abcam

Product datasheet

Anti-Ki67 antibody [EPR3610] - BSA and Azide free ab209897



Recombinant

RabMAb

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Overview

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

DescriptionRabbit monoclonal [EPR3610] to Ki67 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), IHC-P, WB, ICC/IF

Species reactivity Reacts with: Human

Does not react with: Mouse, Rat

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa and ramos cell lysates. IHC-P: Human tonsil, colon, ovarian carcinoma, squamous cell

carcinoma of cervix and colonic adenocarcinoma tissues. ICC/IF: HeLa, HT-29 cells, KI67-HAP1

cells (WT and KO). Flow Cyt (intra): Ramos cells, HAP1 cells.

General notes ab209897 is the carrier-free version of <u>ab92742</u>.

This product is not suitable for xenograft experiments. For further information please contact our

Customer Services team.

Our $\underline{\textbf{carrier-free}}$ 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 $^{\circledR}$ 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

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- Animal-free production

For more information see here.

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

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

ClonalityMonoclonalClone numberEPR3610

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab209897 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
Flow Cyt (Intra)		1/100 - 1/150. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
IHC-P	**** <u>(1)</u>	1/500 - 1/1000. See <u>IHC antigen retrieval protocols</u> . For unpurified use at 1/500 - 1/1000.
WB		1/5000. Predicted molecular weight: 359 kDa. For unpurified use at 1/500 - 1/1000.
ICC/IF		Use a concentration of 1 μ g/ml. If fixing cells in 4% PFA, 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).

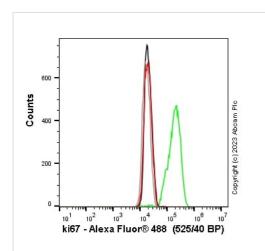
Post-translational modifications

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



Flow Cytometry (Intracellular) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897) 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 MKl67 knockout Hap1 stained with <u>ab92742</u> (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 (<u>ab92742</u>) (1x 10^6 in 100μ I at $0.04~\mu$ g/mI (1/57000)) for 30min at 22° C.

The secondary antibody Goat Anti-Rabbit lgG 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.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ki67 antibody [EPR3610]

- BSA and Azide free (ab209897)

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

Comparison between RNApII-S2P-/low cells and Ki-67- cells

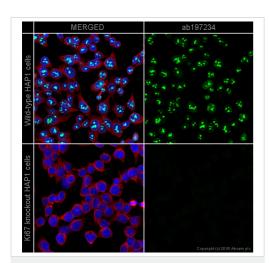
a: Regulation of Ki-67 and RNApll-S2P during proliferation and quiescence in T98G glioblastoma cells. T98G cells were grown in culture medium containing 10% (v/v) fetal bovine serum (FBS), were induced to become guiescent by serum starvation in medium supplemented with 0.5% (v/v) FBS for 14 days, and then were restimulated 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; RNApll-S2P, red; a3, a6, a9). Hematoxylin (blue) was used as a nuclear stain. Ki-67- RNApll-S2P-/low cells (blue cells in the double stained sections) emerged only in the guiescent 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.

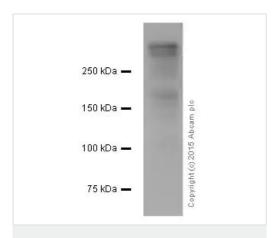
(From Figure S2 of Ishii et al)

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.



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



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

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).

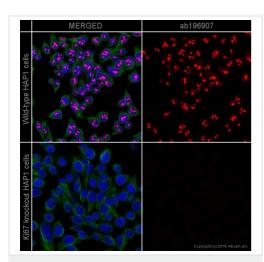
Anti-Ki67 antibody [EPR3610] (ab92742) at 1/5000 dilution (purified) + Ramos (Human Burkitt's lymphoma cell line) cell lysate at 20 µg

Secondary

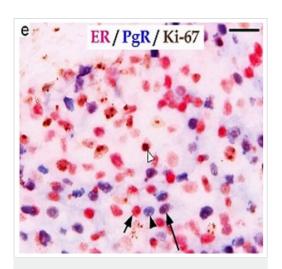
Peroxidase-conjugated goat anti-rabbit lgG, (H+L) at 1/1000 dilution

Predicted band size: 359 kDa **Observed band size:** 395 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ki67 antibody [EPR3610]

- BSA and Azide free (ab209897)

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

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.

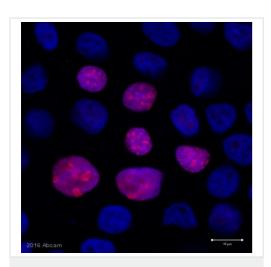
ab196907 staining Ki67 in wild-type HAP1 cells (top panel) and Ki67 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells 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).

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-67⁻ cells were stained red (short arrow), ER⁻ PgR⁺ Ki-67⁻ cells were stained blue (black arrowhead), ER⁺ PgR⁺ Ki-67⁻ cells were stained purple (long arrow), and Ki-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.



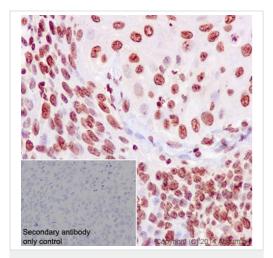
Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

This image is courtesy of an anonymous Abreview.

<u>ab92742</u> 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 lgG polyclonal was used as the secondary antibody at a dilution of 1/200.

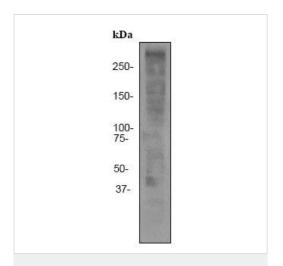
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).



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

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).



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

ab92742 MERGED

DAPI

Ove control 1

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

Anti-Ki67 antibody [EPR3610] (ab92742) at 1/500 dilution (unpurified) + HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

Secondary

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

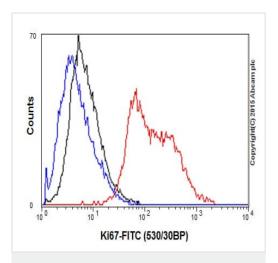
Predicted band size: 359 kDa Observed band size: 395 kDa

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

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 lgG (1/500).

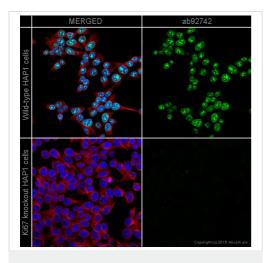
Control 2: <u>ab7291</u> (1/1000) and secondary antibody, <u>ab150077</u>, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500).



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

Intracellular Flow Cytometry analysis of Ramos (Human Burkitt's lymphoma cell line) cells lablling Ki67 with purified <u>ab92742</u> at 1/150 (red). Cells were fixed with 2% paraformaldehyde. An FITC-conjugated goat anti-rabbit lgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabeled control, cells without incubation with primary and secondary antibodies.

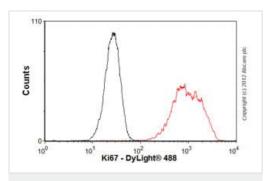
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).



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

<u>ab92742</u> 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 <u>ab92742</u> at 1μg/ml and <u>ab195889</u> 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 lgG (Alexa Fluor® 488) (<u>ab150081</u>) 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 (<u>ab92742</u>).

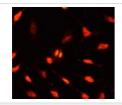


Flow Cytometry (Intracellular) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Overlay histogram showing Ramos (Human Burkitt's lymphoma cell line) cells stained with unpurified <u>ab92742</u> (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 <u>ab92742</u>, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight[®] 488 goat anti-rabbit IgG (H+L) (<u>ab96899</u>) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions.

Acquisition of >5,000 events was performed.

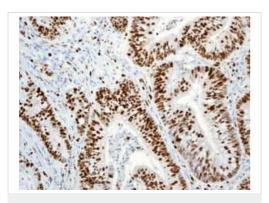
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).



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

Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Ki67 with unpurified <u>ab92742</u> 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).

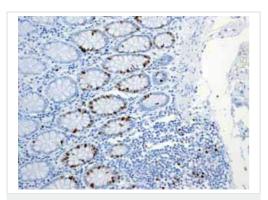


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

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

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.

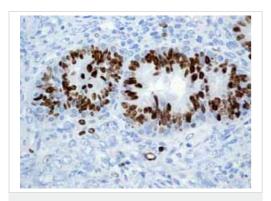


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

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

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.

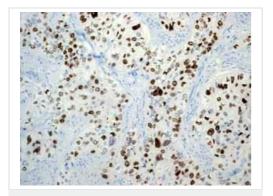


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovarian carcinoma tissue labeling Ki67 with unpurified <u>ab92742</u>.

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.

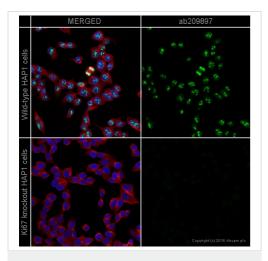


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labeling Ki67 with unpurified <u>ab92742</u>.

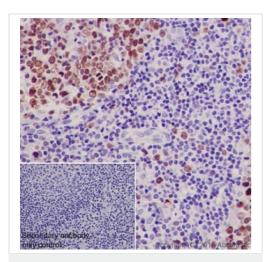
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92742).

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



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

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 1 μ g/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 lgG (Alexa Fluor® 488) (**ab150081**) at 2 μ g/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



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

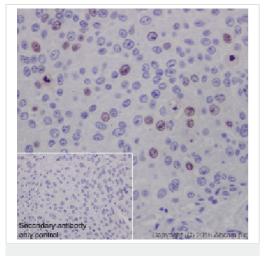
Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling Ki67 with ab209897 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

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 paraffinembedded sections) - Anti-Ki67 antibody [EPR3610] - BSA and Azide free (ab209897)

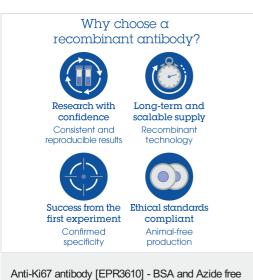
Immunohistochemical analysis of paraffin-embedded Human bladder cancer labeling Ki67 with ab209897 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

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.



(ab209897)

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