abcam

Product datasheet

Anti-Ki67 antibody [EPR3610] ab92742

KO VALIDATED Recombinant RabMAb

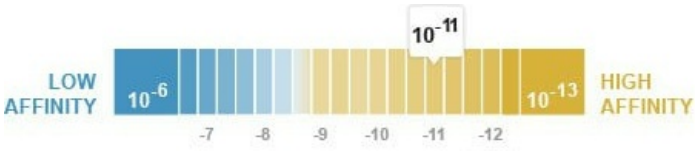
12 Abreviews 312 References 17 Images

Overview

Product name	Anti-Ki67 antibody [EPR3610]
Description	Rabbit monoclonal [EPR3610] to Ki67
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse, Rat
Immunogen	Synthetic peptide within Human Ki67 aa 1050-1150. The exact sequence is proprietary. Database link: **P46013-1**
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, HAP1 cells. Flow Cyt (intra): Ramos cells, HAP1 cells.
General notes	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**. 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Dissociation constant (K_D)	$K_D = 1.24 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3610
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab92742 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 IgG, is suitable for use as an isotype control with this antibody.
WB		1/5000. Predicted molecular weight: 359 kDa. **For unpurified use at 1/500 - 1/1000.**
IHC-P	★★★★☆ **(5)**	1/500 - 1/1000. See **IHC antigen retrieval protocols**. **For unpurified use at 1/500 - 1/1000.**
ICC/IF		Use a concentration of 0.5 - 1 µg/ml. If fixing cells in 4% PFA, it is recommended to permeabilized cells with 0.1% Triton-X for 5 min.

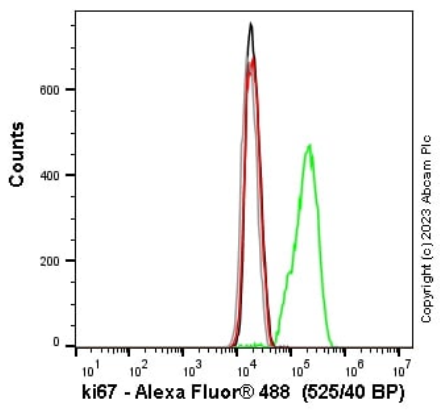
Target

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] (ab92742)

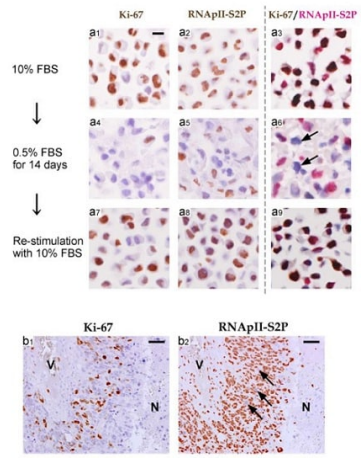
Flow cytometry overlay histogram showing wild-type Hap1 (green line) and MKI67 knockout Hap1 stained with ab92742 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab92742) (1x 10^6 in 100µl at 0.04 µg/ml (1/57000)) for 30min at 22°C.

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

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

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

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] (ab92742)

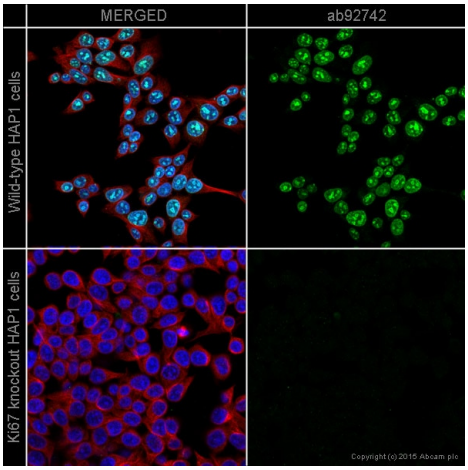
Ishii et al PLoS One. 2016 Jan 22;11(1):e0147366. doi: 10.1371/journal.pone.0147366. eCollection 2016

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

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

Ki67 detected using ab92742.

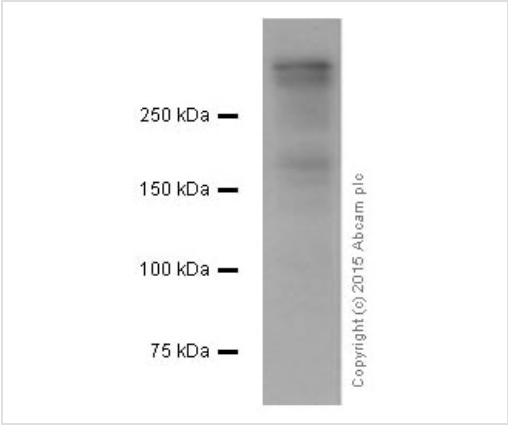
(From Figure S2 of Ishii et al)



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] (ab92742)

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

Alexa Fluor® 488 (**ab197234**) and Alexa Fluor® 647 (**ab196907**) conjugated versions are available for this clone.



Western blot - Anti-Ki67 antibody [EPR3610] (ab92742)

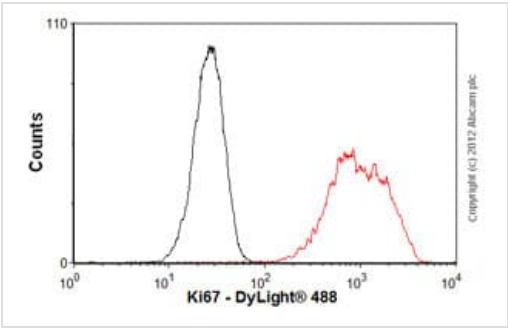
Anti-Ki67 antibody [EPR3610] (ab92742) at 1/5000 dilution (purified) + Ramos (Human Burkitt's lymphoma cell line) cell lysate at 20 µg

Secondary

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

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

Blocking and dilution buffer: 5% NFDM/TBST.

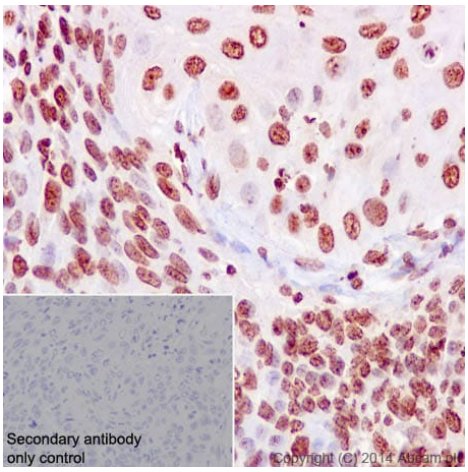


Flow Cytometry (Intracellular) - Anti-Ki67 antibody [EPR3610] (ab92742)

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

Acquisition of >5,000 events was performed.

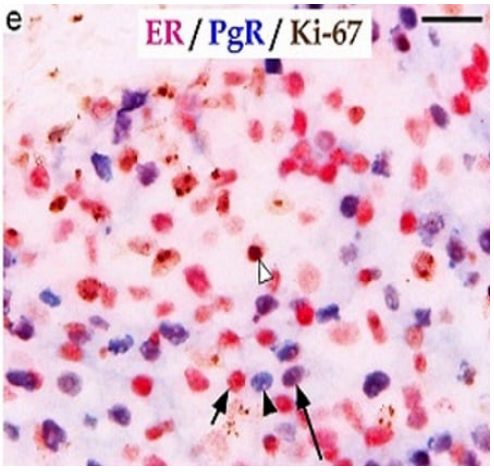
Alexa Fluor®488 (**ab197234**) and Alexa Fluor®647 (**ab196907**) conjugated versions are available for this clone.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] (ab92742)

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).



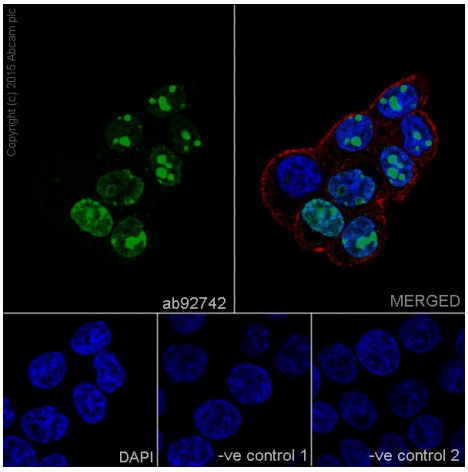
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] (ab92742)

Ishii et al PLoS One. 2016 Jan 22;11(1):e0147366. doi: 10.1371/journal.pone.0147366. eCollection 2016. Fig S4. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Chromogenic triple immunostaining for estrogen receptor (ER), progesterone receptor (PgR), and Ki-67 in breast cancer tissue to verify the triple immunostaining detection method.

Panel e: ER^+ PgR^- $Ki\text{-}67^-$ cells were stained red (short arrow), ER^- PgR^+ $Ki\text{-}67^-$ cells were stained blue (black arrowhead), ER^+ PgR^+ $Ki\text{-}67^-$ cells were stained purple (long arrow), and $Ki\text{-}67^+$ cells were stained brown (white arrowhead). These colors are easily distinguishable. Scale bars, 25 μm.

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

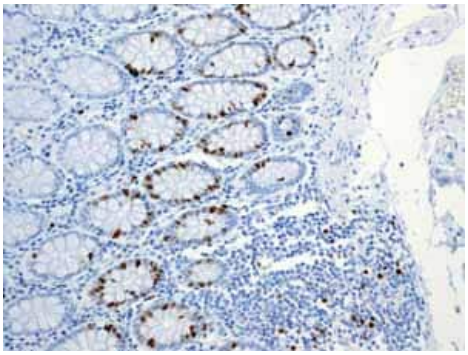


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] (ab92742)

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

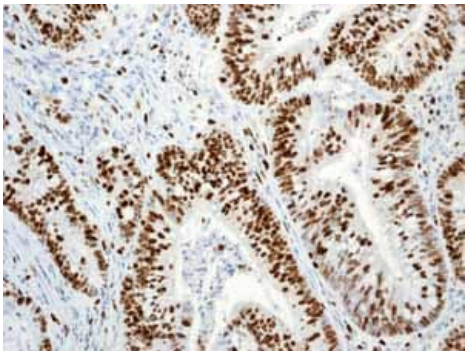
Control 1: primary antibody (1/250) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

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



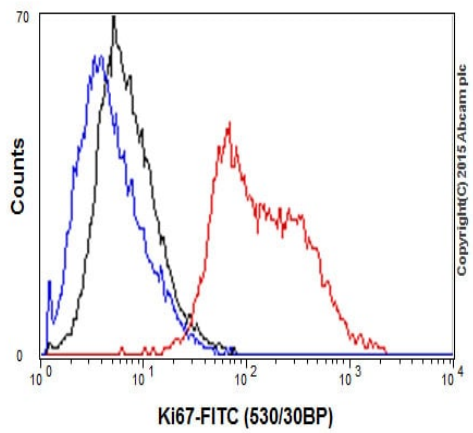
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] (ab92742)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human normal colon tissue labeling Ki67 with unpurified ab92742. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



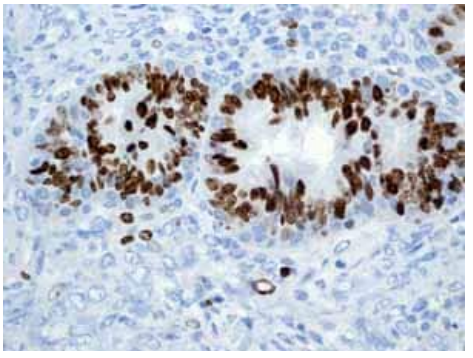
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] (ab92742)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colonic adenocarcinoma tissue labeling Ki67 with unpurified ab92742. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



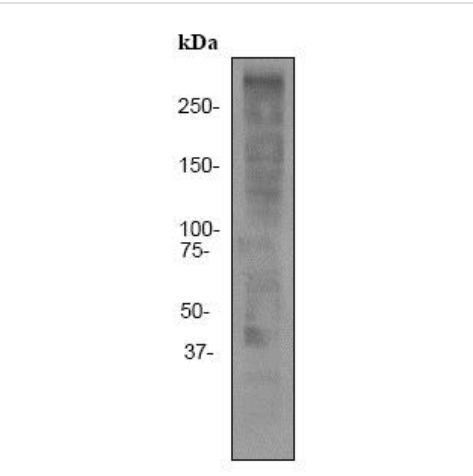
Flow Cytometry (Intracellular) - Anti-Ki67 antibody [EPR3610] (ab92742)

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] (ab92742)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovarian carcinoma tissue labeling Ki67 with unpurified ab92742. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.

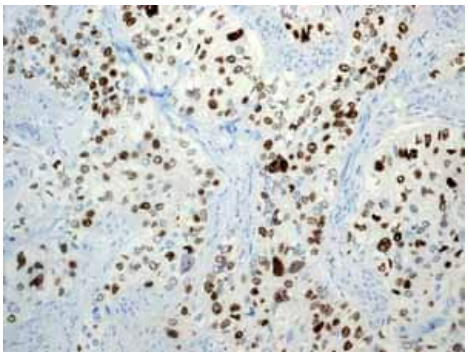


Western blot - Anti-Ki67 antibody [EPR3610] (ab92742)

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

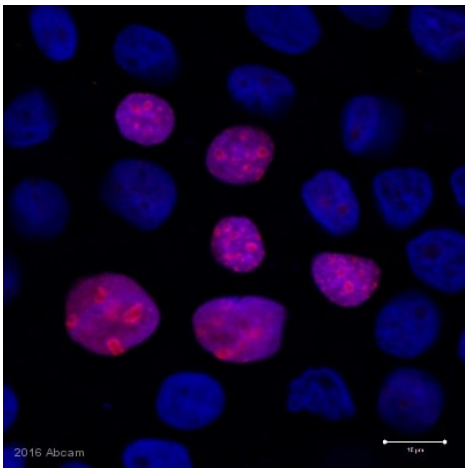
Secondary
HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [EPR3610] (ab92742)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labeling Ki67 with unpurified ab92742. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.

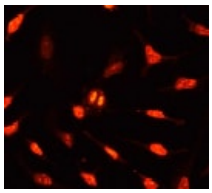


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] (ab92742)

This image is courtesy of an anonymous Abreview.

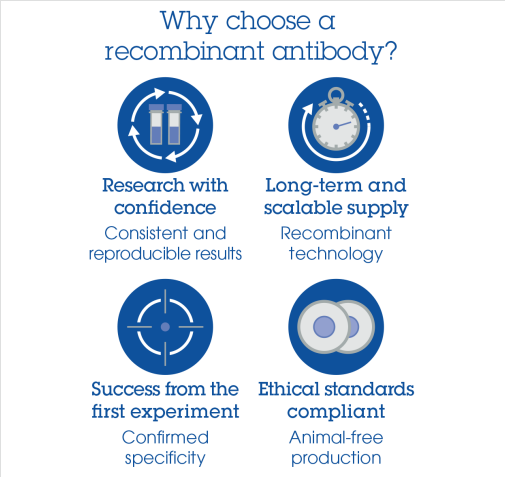
ab92742 staining Ki67 in human adenocarcinoma cells by ICC (Immunocytochemistry).

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



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [EPR3610] (ab92742)

Immunocytochemistry analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Ki67 with unpurified ab92742 at a dilution of 1/250.



Anti-Ki67 antibody [EPR3610] (ab92742)

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