

Product datasheet

Anti-Ki67 antibody ab15580

KO VALIDATED

★★★★★ 204 Abreviews 1881 References 15 Images

Overview

Product name	Anti-Ki67 antibody
Description	Rabbit polyclonal to Ki67
Host species	Rabbit
Tested applications	Suitable for: IHC-P, ICC
Species reactivity	Reacts with: Mouse, Human Predicted to work with: Rat, Sheep, Rabbit, Horse, Cow, Dog, Pig, Monkey, Chinese hamster, Common marmoset, Syrian hamster 
Immunogen	Synthetic peptide corresponding to Human Ki67 aa 1200-1300 (internal sequence) conjugated to keyhole limpet haemocyanin. Database link: P46013 (Peptide available as ab15581)
General notes	<p>Ab15580 is batch tested in ICC/IHC. A variability in IHC-Fr performance can occur with this antibody but we can guarantee consistency in IHC-P. Some customers have successfully used ab15580 in Western Blot. Higher molecular weight proteins like Ki67 may be more difficult to detect in WB. We recommend several potential optimisation steps: loading higher amounts of protein (20 µg and above), using lower percentage gels and/or Tris-Acetate gels, increasing antioxidant to maintain protein reduction, decreasing methanol and increasing SDS in the transfer buffer, and increasing time and voltage of transfer. Larger proteins can be subject to degradation more than smaller proteins so lower molecular weight bands may be present. For further information or support please contact our Scientific Support Team.</p> <p>Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.</p> <p>Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.</p> <p>We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.</p> <p>In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.</p> <p>We are also updating the applications & species that this product has been “predicted to work</p>

with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS
	Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab15580** 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
IHC-P	★★★★☆	Use a concentration of 0.1 - 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC	★★★★★	Use a concentration of 0.5 - 1 µg/ml. For nuclear proteins, fixing cells in 4% PFA (20 min, room temp) is recommended. It is recommended to incubate cells with 0.1% Triton-X for 5 min to detect nuclear antigen. Positive Control: HeLa and HAP1 cells / Rt cardiomyocytes

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
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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).

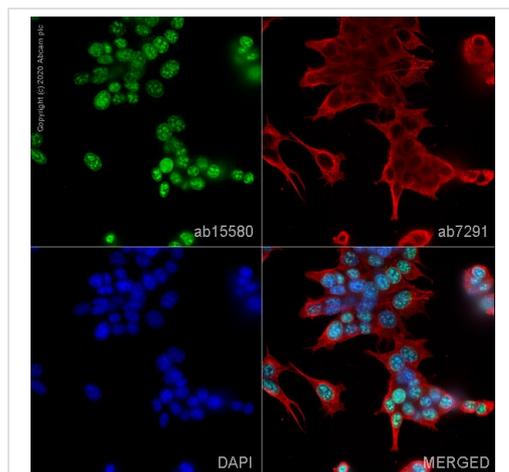
Post-translational modifications

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

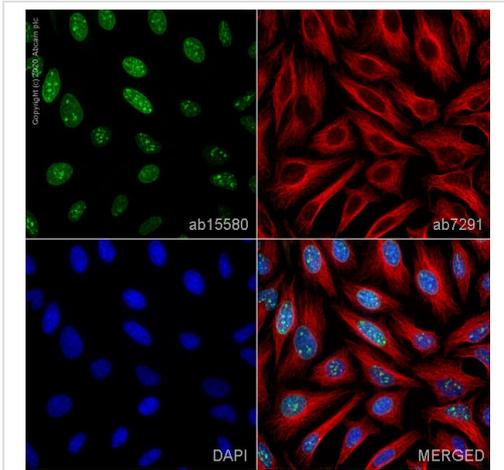


Immunocytochemistry - Anti-Ki67 antibody (ab15580)

ab15580 staining Ki67 in Mef1 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-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 overnight at 4°C with ab15580 at 0.5 µg/ml and [ab7291](#), Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with [ab150081](#), Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and [ab150120](#), Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a single confocal section is shown.

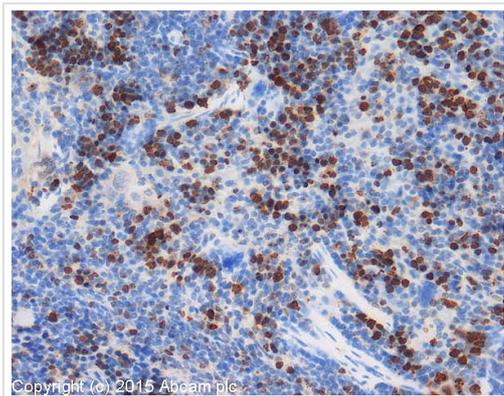


Immunocytochemistry - Anti-Ki67 antibody (ab15580)

ab15580 staining Ki67 in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-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 overnight at 4°C with ab15580 at 0.5 µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

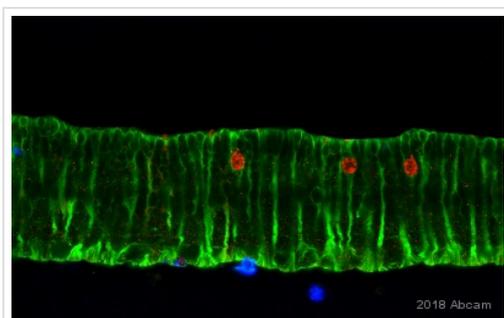
Also suitable in cells fixed with 4% paraformaldehyde (10 min).

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody (ab15580)

IHC image of ab15580 staining in mouse spleen formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol B. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) for 20 mins. The section was then incubated with ab15580, 5µg/ml, for 15 mins at room temperature. A goat anti-rabbit biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

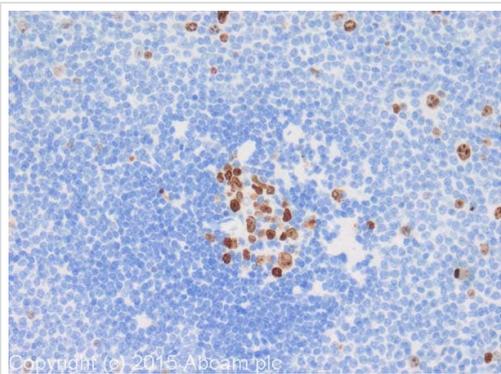


Immunocytochemistry - Anti-Ki67 antibody (ab15580)

Paraformaldehyde-fixed Rabbit cell (Retina) labeling Ki67 (Green) using ab15580 at 1/200 dilution followed by a Donkey anti-rabbit Alexa Fluor® 568 secondary antibody in ICC analysis. Normal Donkey serum was used as the blocking agent for 15 hours at 4°C.

Tissue was immersion fixed in 4% paraformaldehyde overnight at 4 degrees Celsius. Tissue was then embedded in 10% agarose and section at 100 microns. Sections were placed in 2N HCL for 1 hour before commencing immunocytochemistry. Ki-67 (dividing cells red).

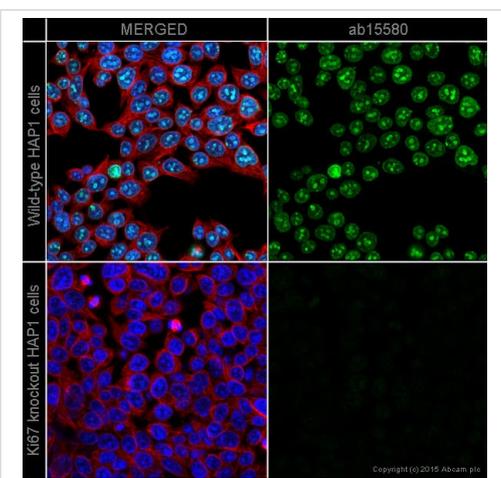
This image was courtesy of an AbReview from Mr. Gabriel Luna



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody (ab15580)

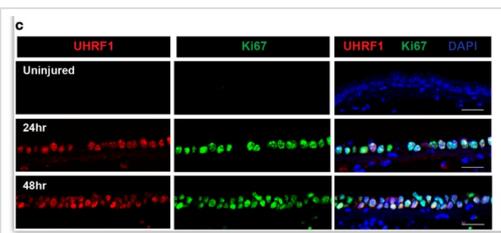
IHC image of Ki67 staining in human spleen formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) for 20 mins. The section was then incubated with ab15580, 1µg/ml, 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.



Immunocytochemistry - Anti-Ki67 antibody (ab15580)

ab15580 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 ab15580 at 1µg/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 anti-rabbit IgG Alexa Fluor® 488 (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

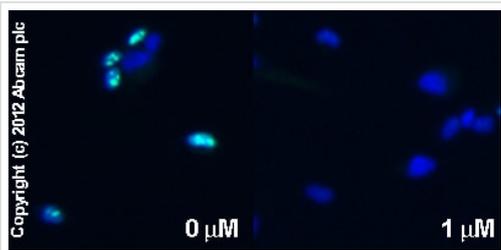


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody (ab15580)

Image courtesy of Xiang H. et al. Cell Discov. 2017; 3: 17019. doi: 10.1038/celldisc.2017.19 Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>.

Confocal images of mouse trachea epithelium collected at steady state, 24 and 48 h after SO₂ injury. Tissue sections were co-stained with UHRF1 and Ki67, a proliferation marker.

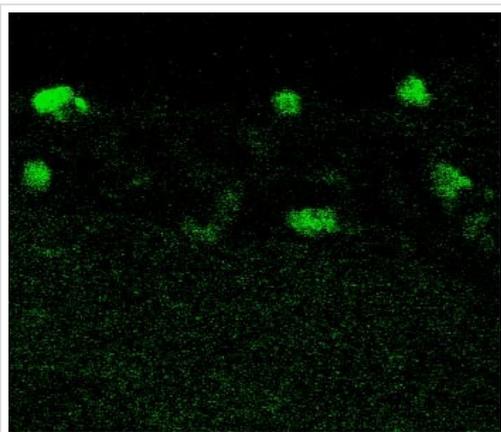
ab15580 was used to stain Ki67 at a dilution of 1:1 000



Immunocytochemistry - Anti-Ki67 antibody
(ab15580)

ab15580 staining Ki67 in SK-N-SH cells treated with NADA (N-Arachidonyldopamine) (ab120099), by ICC. Decrease in Ki67 expression correlates with increased concentration of NADA (N-Arachidonyldopamine), as described in literature.

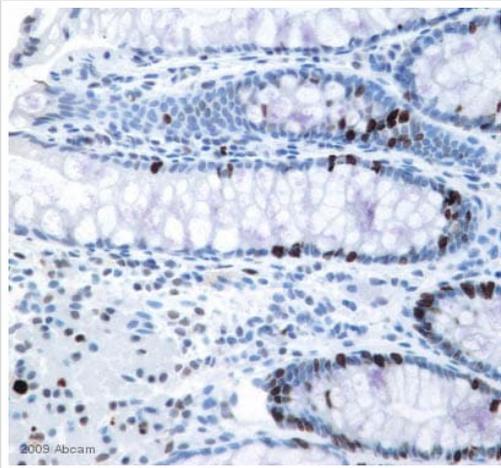
The cells were incubated at 37°C for 10 minutes in media containing different concentrations of ab120099 (NADA (N-Arachidonyldopamine)) in ethanol, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab15580 (1 μg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A goat anti-rabbit DyLight 488 secondary antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Immunocytochemistry - Anti-Ki67 antibody
(ab15580)

Image courtesy of Julien Laffaire, Laboratoire de Neurobiologie, ESPCI, Paris, France

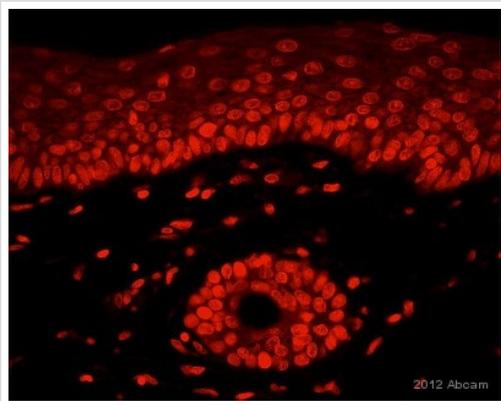
Fluorescent confocal microscopy (20x) of mouse (P0) olfactory bulb, outer glomeruli layer, showing Ki67 immunoreactivity (ab15580; 1/1000; overnight at RT, 0.25% TX-100 no blocking step) using a secondary goat anti-rabbit fluorescent antibody (Alexa Fluor 488; 1/300 2h at RT).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody (ab15580)

This image is courtesy of an anonymous Abreview

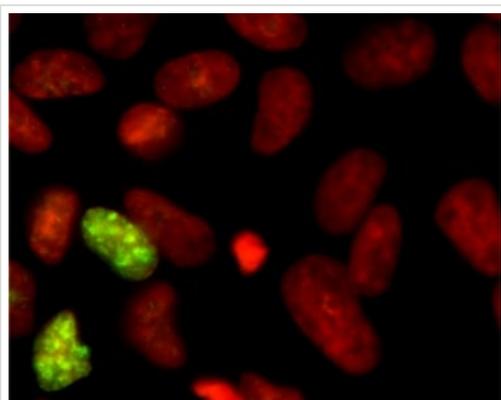
ab15580 staining Ki67-Proliferation Marker in human colon tissue sections by IHC-P (formaldehyde-fixed paraffin-embedded sections). Tissue samples were fixed with formaldehyde and blocked with 10% goat serum for 30 minutes at 25°C. Antigen retrieval was by heat mediation in Target Retrieval Solution. Samples were incubated with primary antibody 1/5000 (TBST) for 1 hour at 25°C.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody (ab15580)

This image is courtesy of an anonymous Abreview

ab15580 staining Ki67 - Proliferation Marker in Human skin tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and blocked with 4% serum for 30 minutes at 25°C; antigen retrieval was by heat mediation in a citrate buffer (pH 6.0). Samples were incubated with primary antibody (5 µg/ml in blocking buffer) for 16 hours at 4°C. A Texas Red® Goat anti-rabbit IgG polyclonal (1/100) was used as the secondary antibody.

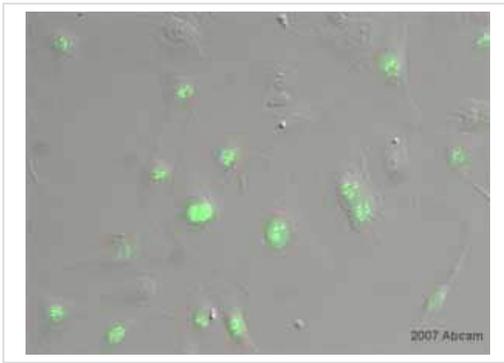


Immunocytochemistry - Anti-Ki67 antibody (ab15580)

This image is courtesy of Kirk McMannus, University of British Columbia

SK-N-SH cells were permitted to grow to confluency, then serum starved for 48 hours and predominantly driven into G0. The cells were then paraformaldehyde fixed and immunofluorescently labelled with anti-Ki67 (ab15580) at a dilution of 1/1000. The majority of the cells show little or no Ki67 staining, indicating they are in G0 arrest (red cells). Two cells however show strong nucleolar Ki67 staining indicating they are still cycling (green cells). The DNA is stained with DAPI and is shown in red. The Ki67 staining is shown in green. x 63 magnification.

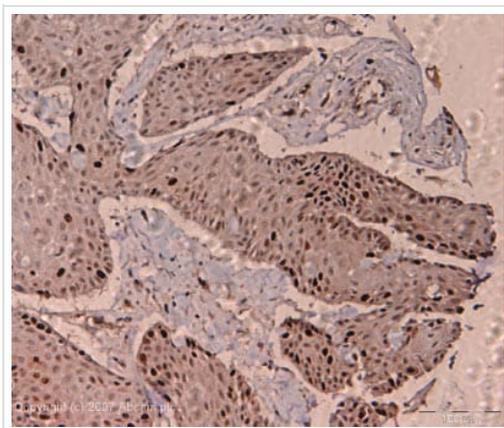
Similar results were seen with an asynchronous population of HeLa cells. The Ki67 staining was localised to the periphery of the nucleoli and throughout the nucleoplasm of proliferating cells. (This data is not shown but is available upon request).



Immunocytochemistry - Anti-Ki67 antibody
(ab15580)

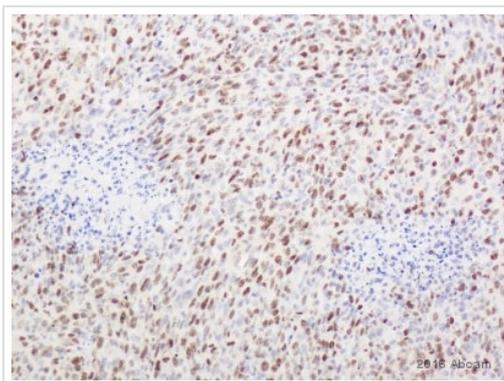
This image is courtesy of an Abreview submitted by Dr Jose Javier Martin De Llano

ab15580 at 1/50 staining Human umbilical artery endothelial cells by ICC. The tissue was paraformaldehyde fixed and blocked before permeabilization with saponin and incubation with the antibody for 16 hours. A FITC conjugated goat anti-rabbit IgG was used as the secondary. The merged image shows those cells expressing Ki67 from the total number of exponential cells.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody (ab15580)

IHC image of ab15580 stained human skin carcinoma FFPE section. Section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30 seconds at 125°C. Section was incubated with ab15580 at a dilution of 1:200 for 1h at room temperature and detected using an HRP conjugated polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody (ab15580)

This image is courtesy of an abreview submitted by Jim Manavis

Immunohistochemistry analysis of Formaldehyde-fixed paraffin-embedded mouse tumour tissue sections labelling Ki67 with ab15580 at 1/2000. The secondary antibody was biotin conjugated goat polyclonal vector at a dilution of 1/250.

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