abcam

Product datasheet

Anti-Ki67 antibody ab15580

敲除验证

223 Abreviews 3592 References 16 图像

概述

产品名称	Anti-Ki67抗体
描述	**兔多克隆抗体**to Ki67
宿主	Rabbit
特异性	From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help.
经测试应用	**适用于:** IHC-P, ICC/IF
种属反应性	**与反应:** Mouse, Human **预测可用于:** Rat, Sheep, Rabbit, Horse, Cow, Dog, Pig, Monkey, Chinese hamster, Common marmoset, Syrian hamster
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab15581**)
常规说明	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. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. 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, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	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.
存储溶液	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.
纯度	Immunogen affinity purified
克隆	多克隆
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab15580于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

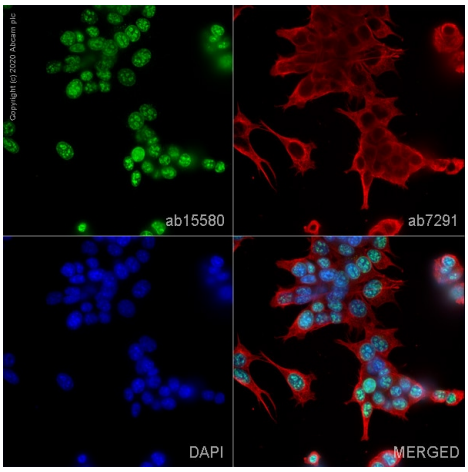
应用	Ab评论	说明
IHC-P	★★★★☆ (68)	Use a concentration of 0.1 - 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF	★★★★★ (8)	Use a concentration of 0.5 - 1 µg/ml. If fixing cells in 4% PFA (20 min, room temp), it is recommended to permeabilized cells with 0.1% Triton-X for 5 min. Positive Control: HeLa and HAP1 cells

靶标

功能	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 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.
序列相似性	Contains 1 FHA domain. Contains 16 K167R repeats. Contains 1 PP1-binding domain.

发展阶段	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).
翻译后修饰	Phosphorylated. Hyperphosphorylated in mitosis (PubMed:10502411, PubMed:10653604). Hyperphosphorylated form does not bind DNA.
细胞定位	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).

图片

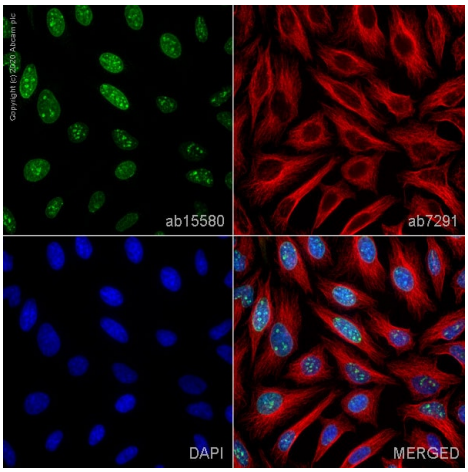


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody (ab15580)

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.

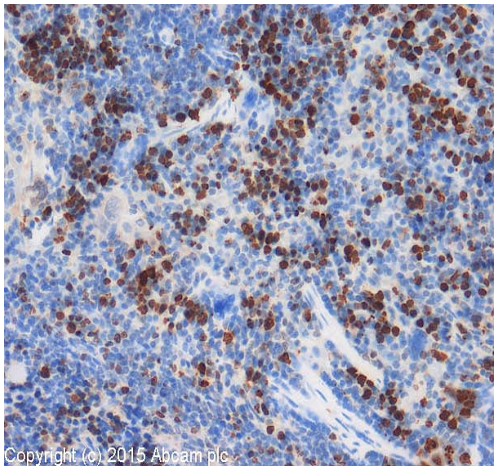


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody (ab15580)

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).

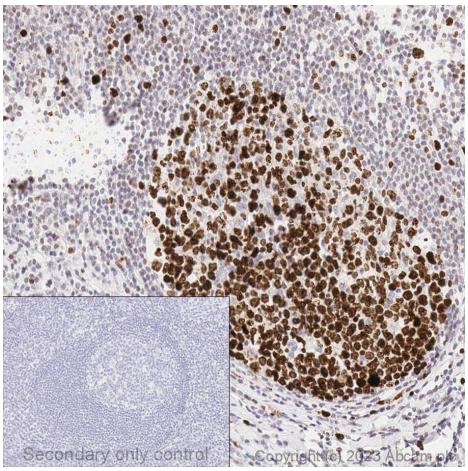
Also suitable in cells fixed with 4% paraformaldehyde (10 min).

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.



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

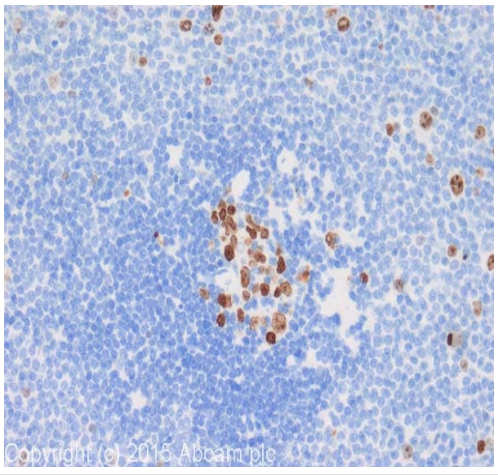
Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling Ki67 with ab15580 at a concentration of 0.5 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with ULTRA cell conditioning solution (CC1 pH8.5). ab15580 anti Ki67 antibody was incubated at 37°C for 16min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control

Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody (ab15580)

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

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 4ºC.

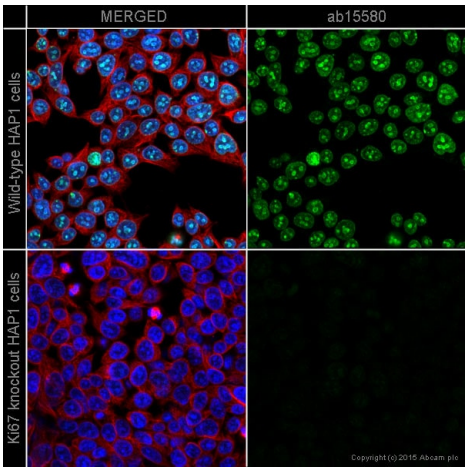
Tissue was immersion fixed in 4% paraformaldehyde overnight at 4 degrees Celsius. Tissue was then embedded in 10% agarose and section at 100 microns. Sections were placed in 2N HCL for 1 hour before commencing immunocytochemistry. Ki-67 (dividing cells red).



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

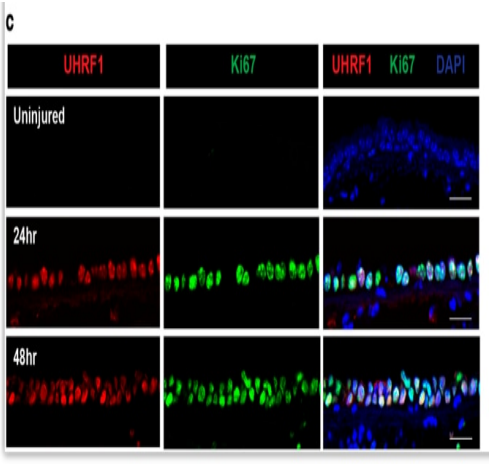
IHC image of Ki67 staining in human spleen formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) for 20 mins. The section was then incubated with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

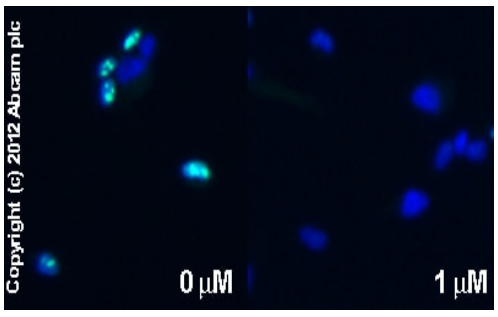


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody (ab15580)

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 SO_2 injury. Tissue sections were co-stained with UHRF1 and Ki67, a proliferation marker.

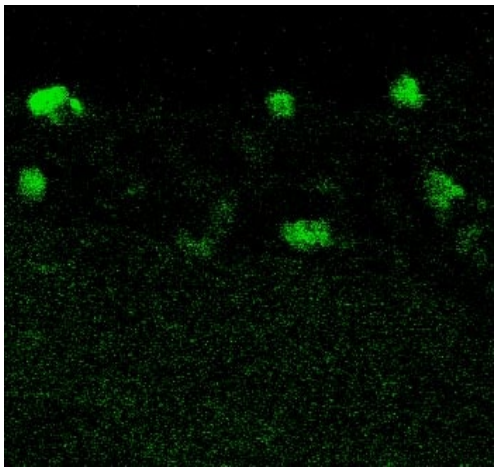
ab15580 was used to stain Ki67 at a dilution of 1:1 000



Immunocytochemistry/ Immunofluorescence - 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**

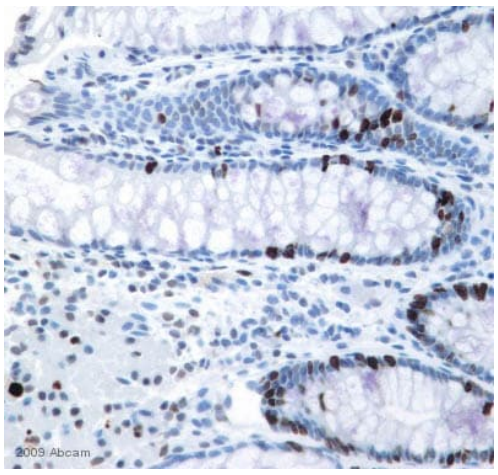
antibody (**ab96899**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody (ab15580)

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

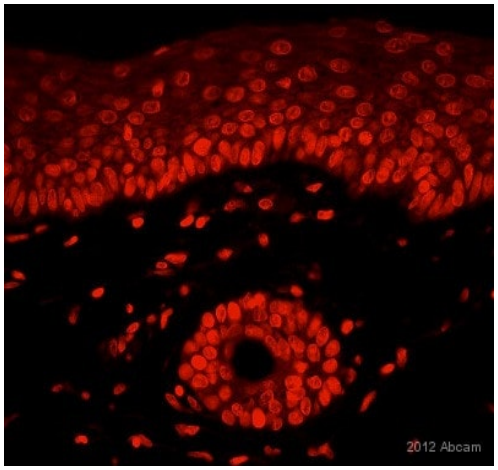
Fluorescent confocal microscopy (20x) of mouse (P0) olfactory bulb, outer glomeruli layer, showing Ki67 immunoreactivity (ab15580; 1/1000; overnight at RT, 0.25% TX-100 no blocking step) using a secondary goat anti-rabbit fluorescent antibody (Alexa Fluor 488;1/300 2h at RT.



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

This image is courtesy of an anonymous Abreview

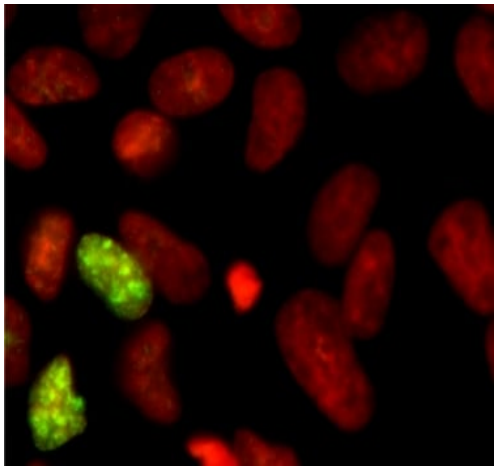
ab15580 staining Ki67-Proliferation Maker in human colon tissue sections by IHC-P (formaldehyde-fixed paraffin-embedded sections). Tissue samples were fixed with formaldehyde and blocked with 10% goat serum for 30 minutes at 25°C. Antigen retrieval was by heat mediation in Target Retrieval Solution.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

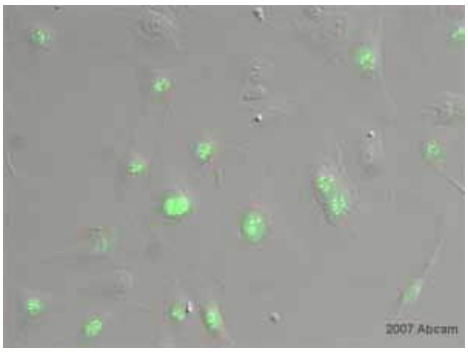


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody (ab15580)

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.

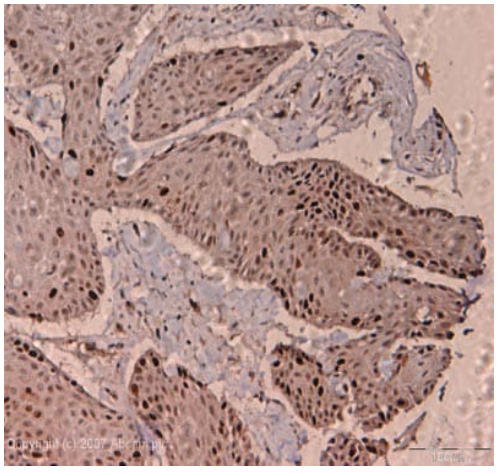
Similar results were seen with an asynchronous population of HeLa cells. The Ki67 staining was localised to the periphery of the nucleoli and throughout the nucleoplasm of proliferating cells. (This data is not shown but is available upon request).



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody (ab15580)

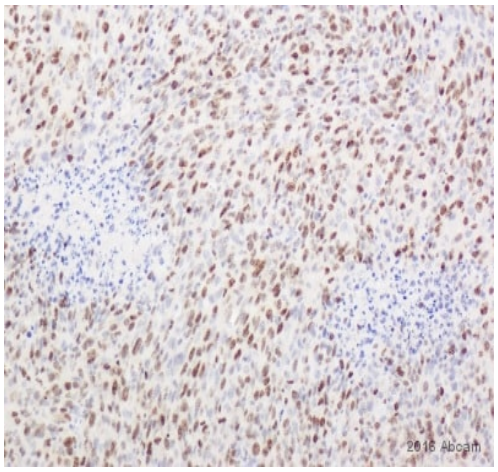
This image is courtesy of an Abreview submitted by Dr Jose Javier Martin De Llano

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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