abcam

Product datasheet

Alexa Fluor® 488 Anti-Ki67 antibody [SP6] ab281847

Recombinant RabMAb

1 References 5 Images

Overview

Product name Alexa Fluor® 488 Anti-Ki67 antibody [SP6]

Alexa Fluor® 488 Rabbit monoclonal [SP6] to Ki67 **Description**

Host species Rabbit

Conjugation Alexa Fluor® 488. Ex: 495nm, Em: 519nm

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

Species reactivity Reacts with: Mouse, Human

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

Positive control IHC-P: Human and mouse colon tissues. ICC: HeLa cells. Flow Cyt (Intra): HeLa 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**.

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outlicensing@thermofisher.com.

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. Store In the Dark.

Storage buffer pH: 7.2

Preservative: 0.02% Sodium azide

Constituents: 30% Glycerol (glycerin, glycerine), 1% BSA, 68.98% PBS

Purity Protein A purified

Clonality Monoclonal

Clone numberSP6IsotypeIgG1

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab281847 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		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/50.
Flow Cyt (Intra)		1/5000.

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,

Post-translational modifications

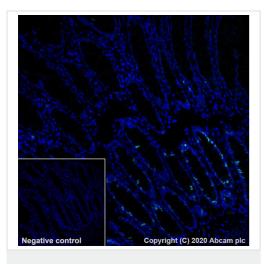
Cellular localization

PubMed:8799815).

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

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

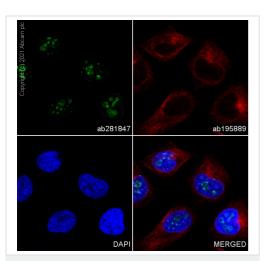


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Alexa Fluor® 488 Anti-Ki67 antibody [SP6] (ab281847)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Ki67 with ab281847.

Positive staining on human colon. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with ab281847 at 1/50 dilution (shown in green). Nuclear DNA was labeled with DAPI (shown in blue). The section was then mounted using Fluoromount[®]. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

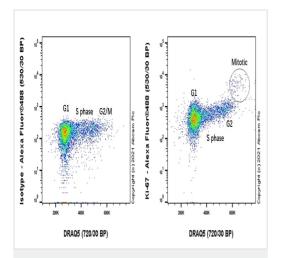
Heat mediated antigen retrieval using $\underline{ab93684}$ (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Anti-Ki67 antibody [SP6] (ab281847)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labelling Ki67 with ab281847 at 1/50 dilution (10.0 µg/ml) (Green).

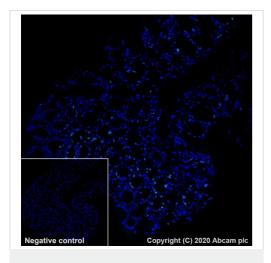
Confocal image showing nucleolar staining in HeLa cells. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody
Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Anti-Ki67 antibody [SP6] (ab281847)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (Human cervix adenocarcinoma cell) cells labelling Ki67 with ab281847 at 1/5000 dilution (0.01 μ g) (Right) compared with a Rabbit monoclonal isotype control - Alexa Fluor 488 (Left) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Cells were co-stained with DRAQ5 to differentiate cell cycle phase.

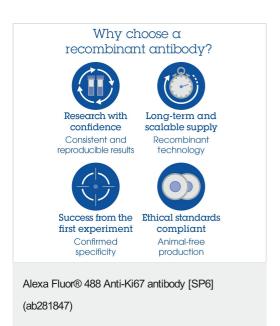


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Alexa Fluor® 488 Anti-Ki67 antibody [SP6] (ab281847)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Ki67 with ab281847.

Positive staining on mouse colon. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with ab281847 at 1/50 dilution (shown in green). Nuclear DNA was labeled with DAPI (shown in blue). The section was then mounted using Fluoromount®. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



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