abcam

Product datasheet

Anti-Ki67 antibody [SP6], prediluted ab21700





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Overview

Product name Anti-Ki67 antibody [SP6], prediluted

Description Rabbit monoclonal [SP6] to Ki67, prediluted

Host species Rabbit

Specificity ab21700 recognises Ki67.

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

Species reactivity Reacts with: Mouse. Human

Predicted to work with: Rat

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

Positive control WB: Ramos and HeLa cell lysates. IHC-P: Human tonsil and thymus tissue; Human endometrial

and breast adenocarcinoma tissue. ICC/IF: HeLa and HAP1 cells. Flow Cyt (intra): HAP1 cells.

General notes This product was switched from hybridoma to recombinant format on 25th October 2019.

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**.

This product is FOR RESEARCH USE ONLY. For commercial use, please contact

partnerships@abcam.com.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C.

Storage buffer pH: 7.60

Preservative: 0.1% Sodium azide

Constituents: Tris buffered saline, 1% BSA

Inert stabilizer

Purity Protein A purified

Clonality Monoclonal

Clone number SP6
Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab21700 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
WB		Use at an assay dependent concentration. Predicted molecular weight: 358 kDa.
IHC-P	★★★★★ (2)	1/1. Antigen retrieval: heat mediated antigen retrieval with sodium citrate buffer (pH 6.0)
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF	**** (1)	Use at an assay dependent concentration. Use at an assay dependent concentration. If fixing cells in 4% PFA (20 min, room temp), 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,

Post-translational modifications

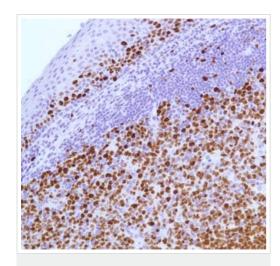
Cellular localization

PubMed:8799815).

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

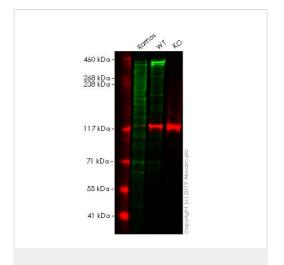
Chromosome. Nucleus. Nucleus, nucleolus. Associates with the surface of the mitotic chromosome, the perichromosomal layer, and covers a substantial fraction of the mitotic chromosome surface (PubMed:27362226). Associates with satellite DNA in G1 phase (PubMed:9510506). Binds tightly to chromatin in interphase, 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) - Anti-Ki67 antibody [SP6], prediluted (ab21700)

Immunohistochemical analysis of formalin-fixed, paraffin-embedded Human tonsil tissue, staining Ki67 with ab21700.



Western blot - Anti-Ki67 antibody [SP6], prediluted (ab21700)

All lanes: Anti-Ki67 antibody [SP6] (ab16667) at 1/100 dilution

Lane 1: Ramos cell lysate

Lane 2: Wild-type HeLa cell lysate

Lane 3: MKI67 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

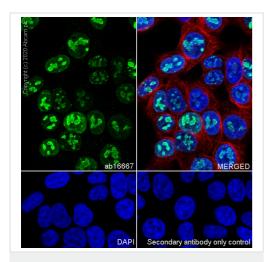
Performed under reducing conditions.

Predicted band size: 358 kDa **Observed band size:** 359 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab16667).

Lanes 1 - 3: Merged signal (red and green). Green - <u>ab16667</u> observed at 359 kDa. Red - loading control, <u>ab130007</u> observed at 125 kDa.

<u>ab16667</u> was shown to react with Ki67 in wild-type HeLa cells. Loss of signal was observed when knockout cell line <u>ab255407</u> (knockout cell lysate <u>ab263762</u>) was used. Wild-type and Ki67 knockout samples were subjected to SDS-PAGE. <u>ab16667</u> and Anti-Vinculin antibody [VIN-54] (<u>ab130007</u>) were incubated overnight at 4°C at 1 in 100 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

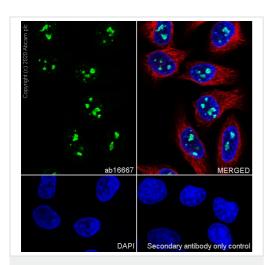


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6], prediluted (ab21700)

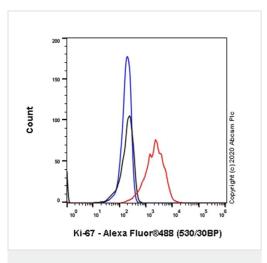
This data was developed using <u>ab16667</u>, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 100% methanol-fixed, None permeabilized parental HAP1 cells labelling Ki67 with <u>ab16667</u> at 1/1000 dilution, followed by <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (2 μg/mL) (Green). Confocal image showing nucleolar staining in parental HAP1cell line <u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 μg/mL) (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) at 1000 dilution (2 μ g/mL).



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [SP6], prediluted (ab21700)



Flow Cytometry (Intracellular) - Anti-Ki67 antibody [SP6], prediluted (ab21700)

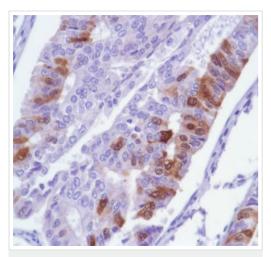
This data was developed using <u>ab16667</u>, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 100% methanol-fixed, None permeabilized HeLa cells labelling Ki67 with <u>ab16667</u> at 1/1000 dilution, followed by <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (2 μg/mL) (Green). Confocal image showing nucleolar staining in HeLa cell line <u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 μg/mL) (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) at 1000 dilution (2 μ g/mL).

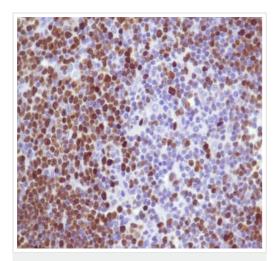
This data was developed using <u>ab16667</u>, the same antibody clone in a different buffer formulation.

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized parental HAP1 (Wildtype control Human chronic myelogenous leukemia near-haploid cell line) cells labelling Ki67 with ab16667 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



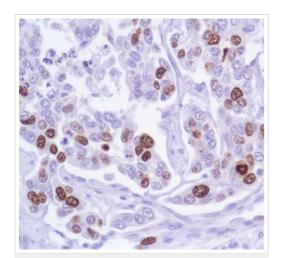
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ki67 antibody [SP6], prediluted (ab21700)

Immunohistochemical analysis of formalin-fixed, paraffin-embedded Human endometrial adenocarcinoma tissue, staining Ki67 with ab21700.



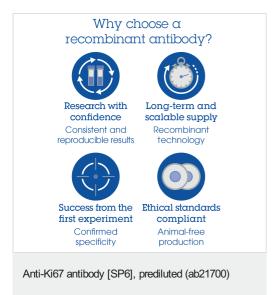
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ki67 antibody [SP6], prediluted (ab21700)

Immunohistochemical analysis of formalin-fixed, paraffin-embedded Human thymus tissue, staining Ki67 with ab21700.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ki67 antibody [SP6], prediluted (ab21700)

Immunohistochemical analysis of formalin-fixed, paraffin-embedded Human breast adenocarcinoma tissue, staining Ki67 with ab21700.



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