abcam

Product datasheet

Anti-Ki67 antibody [37C7-12] ab245113

KO VALIDATED Recombinant

★★★★★ 2 Abreviews 17 References 7 Images

Overview

Product name	Anti-Ki67 antibody [37C7-12]
Description	Mouse monoclonal [37C7-12] to Ki67
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, IHC-P **Unsuitable for:** WB
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human breast carcinoma and tonsil tissue. ICC/IF: Hap1-Ki67 cells Flow Cyt (intra): HAP1 cells.
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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**.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
Purity	Protein A purified
Clonality	Monoclonal
Clone number	37C7-12

Isotype	IgG2a
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab245113 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)		Use 0.02µg for 10^6 cells.
ICC/IF	★★★★★ (1)	Use a concentration of 1 - 2 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.
IHC-P		1/800. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for WB.

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.

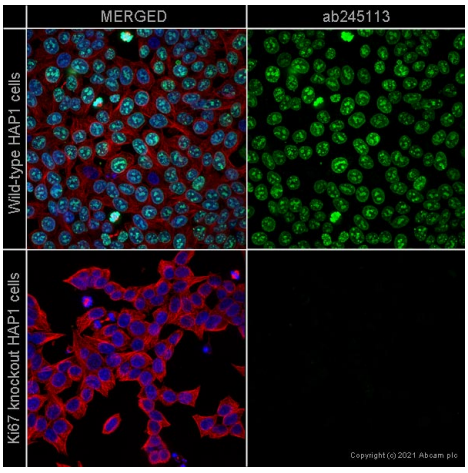
Developmental stage Expression occurs preferentially during late G1, S, G2 and M phases of the cell cycle, while in cells in G0 phase the antigen cannot be detected (at protein level) (PubMed:6206131). Present at highest level in G2 phase and during mitosis (at protein level). In interphase, forms fiber-like 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

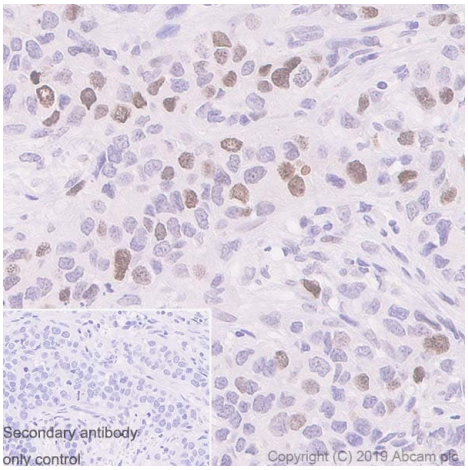


Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [37C7-12] (ab245113)

ab245113 staining Ki67 in Hap1-Ki67 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 ab245113 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

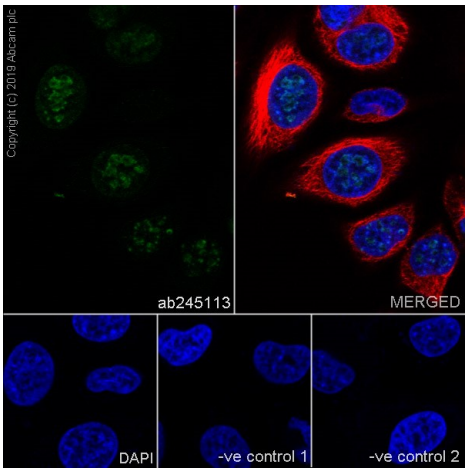
Also suitable in cells fixed with 4% paraformaldehyde (10 min).

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



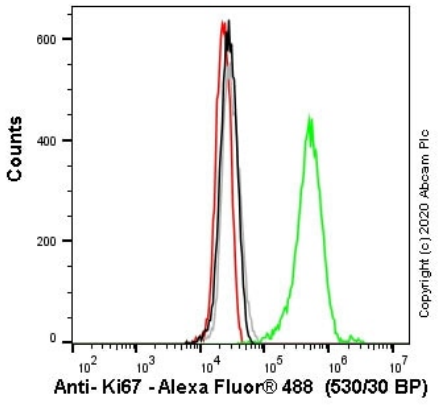
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [37C7-12] (ab245113)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue labeling Ki67 with ab245113 at 1/800 dilution, followed by a ready to use secondary. Nuclear staining on human breast carcinoma tissue is observed. The section was incubated with ab245113 for 30 mins at RT. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, followed by ready to use secondary. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [37C7-12] (ab245113)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Ki67 with ab245113 at 1/100 dilution, followed by Goat Anti-Mouse IgG (Alexa Fluor® 488) (**ab150113**) secondary antibody at 1/1000 dilution (green). Confocal image showing nucleolus staining on HeLa cell line. The nuclear counterstain is DAPI (blue).
Tubulin is detected with **ab179504** Anti-beta IV Tubulin antibody - Microtubule Marker at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 594) (**ab150080**) secondary antibody at 1/1000 dilution (red).
The negative controls are as follows:
-ve control 1: ab245113 at 1/50 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 594) (**ab150080**) secondary antibody at 1/1000 dilution.
-ve control 2: **ab179504** at 1/200 dilution, followed by **ab150113** AlexaFluor®488 Goat anti-Mouse secondary at 1/1000 dilution.



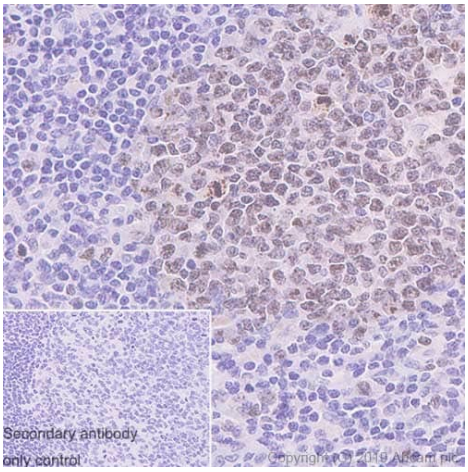
Flow Cytometry (Intracellular) - Anti-Ki67 antibody [37C7-12] (ab245113)

Intracellular Intracellular Flow Cytometry overlay histogram showing wild-type HAP1 (green line) and MKI67 knockout HAP1 cells stained with ab245113 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized 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 (ab245113) ($1x10^6$ in 100 µl at 0.2 µg/ml) for 30 min at 22°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 22°C.

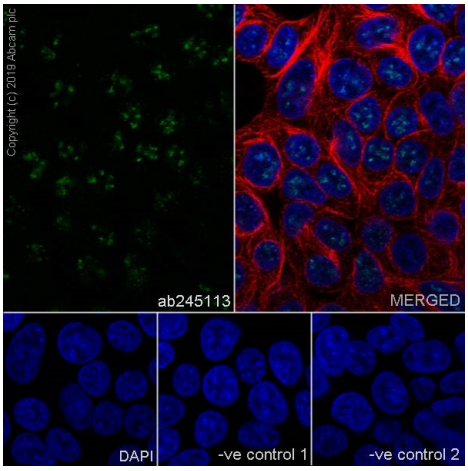
Isotype control antibody was mouse IgG2a&kappa (**ab18413**) used at the same concentration and conditions as the primary antibody (wild-type HAP1 - black line MKI67 knockout HAP1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. This antibody can also be used in HAP1 cells fixed with 80% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ki67 antibody [37C7-12] (ab245113)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling Ki67 with ab245113 at 1/800 dilution, followed by a ready to use secondary. Nuclear staining on human tonsil tissue is observed. The section was incubated with ab245113 for 30 mins at RT. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, followed by ready to use secondary. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-Ki67 antibody [37C7-12] (ab245113)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HAP1 (human chronic myelogenous leukemia near-haploid cell line) cells labeling Ki67 with ab245113 at 1/100 dilution, followed by Goat Anti-Mouse IgG (Alexa Fluor® 488) (**ab150113**) secondary antibody at 1/1000 dilution (green). Confocal image showing nucleolus staining on HAP1 cell line. The nuclear counterstain is DAPI (blue).
Tubulin is detected with **ab179504** Anti-beta IV Tubulin antibody - Microtubule Marker at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 594) (**ab150080**) secondary antibody at 1/1000 dilution (red).
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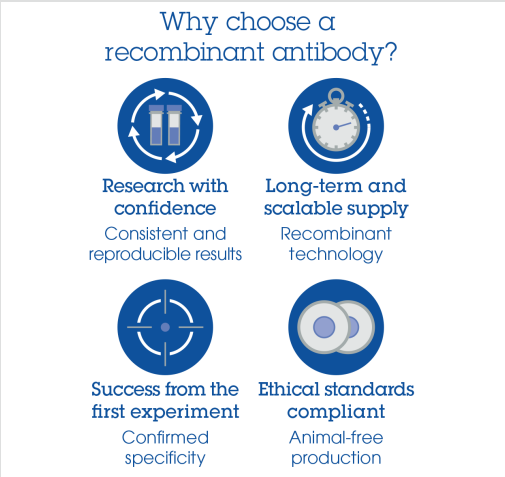

Anti-Ki67 antibody [37C7-12] (ab245113)

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