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Anti-Ki67 Antibody Picoband™ (monoclonal, 5E12)

Catalog # ABO15053

Product Information

Application	IHC, IF, ICC, FC
Primary Accession	P46013
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Ki67 Antibody Picoband™ (monoclonal, 5E12) . Tested in Flow Cytometry, IF, IHC, ICC applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	4288
Other Names	Proliferation marker protein Ki-67, Antigen identified by monoclonal antibody Ki-67, Antigen KI-67, Antigen Ki67, MKI67 (HGNC:7107)
Calculated MW	358694
Application Details	Immunohistochemistry (Paraffin-embedded Section), 2-5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 5E12
Immunogen	E. coli-derived human Ki67 recombinant protein (Position: K2860-I3256).
Purification	Immunogen affinity purified.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	MKI67 (HGNC:7107)

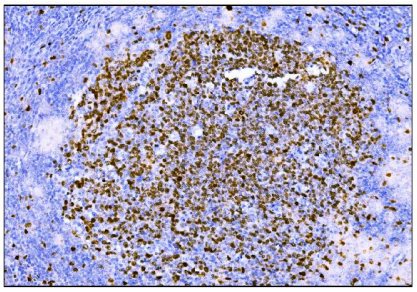
Function	Protein that associates with the surface of mitotic chromosomes and acts both as a chromosome repellent during early mitosis and chromosome attractant during late mitosis (PubMed:27362226, PubMed:32879492, PubMed:35513709, PubMed:39153474). Required to maintain individual mitotic chromosomes dispersed in the cytoplasm following nuclear envelope disassembly (PubMed:27362226). During early mitosis, relocalizes from nucleoli to the chromosome surface where it forms extended brush structures that cover a substantial fraction of the chromosome surface (PubMed:27362226). The MKI67 brush structure 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). During mitotic anaphase, the MKI67 brush structure collapses and MKI67 switches from a chromosome repellent to a chromosome attractant to promote chromosome clustering and facilitate the exclusion of large cytoplasmic particles from the future nuclear space (PubMed:32879492, PubMed:39153474). Mechanistically, dephosphorylation during mitotic exit and simultaneous exposure of a conserved basic patch induce the RNA-dependent formation of a liquid- like condensed phase on the chromosome surface, promoting coalescence of neighboring chromosome surfaces and clustering of chromosomes (PubMed:39153474). Binds premature ribosomal RNAs during anaphase; promoting liquid-liquid phase separation (PubMed:28935370, PubMed:39153474). 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; it is however unclear whether it plays a direct role in chromatin organization or whether it is an indirect consequence of its function in mitotic chromosome (PubMed:24867636).
Cellular Location	Chromosome. Nucleus. Nucleus, nucleolus. Note=During early mitosis, relocalizes from nucleoli to 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)

Background

Ki-67 (Proliferation-related Ki-67 antigen), also known as MKI67 or KIA, is a protein that in humans is encoded by the MKI67 gene. From study of a panel of human-rodent somatic cell hybrids, it has been demonstrated that a gene involved in the expression of the MKI67 antigen is located on chromosome 10. By in situ hybridization, Fonatsch et al. (1991) regionalized the MKI67 gene to chromosome 10q25-qter. By FISH, Traut et al. (1998) mapped the mouse Mki67 gene to chromosome 7F3-F5. Antigen KI-67 is a nuclear protein that is associated with and may be necessary for cellular proliferation. Furthermore it is associated with ribosomal RNA transcription. Inactivation of antigen KI-67 leads to inhibition of ribosomal RNA synthesis.

Images

Figure 1. IHC analysis of Ki67 using anti-Ki67 antibody (M00254-8).
Ki67 was detected in paraffin-embedded section of



human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ki67 Antibody (M00254-8) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

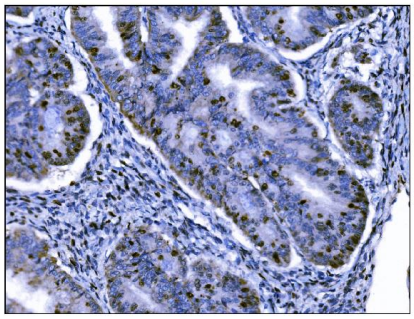


Figure 2. IHC analysis of Ki67 using anti-Ki67 antibody (M00254-8).
Ki67 was detected in paraffin-embedded section of human cervical cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ki67 Antibody (M00254-8) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

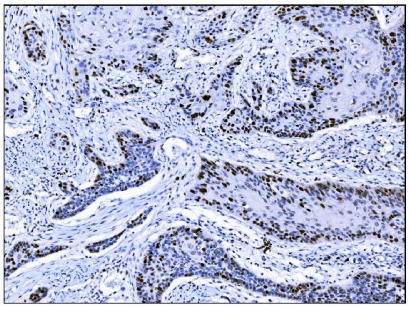


Figure 3. IHC analysis of Ki67 using anti-Ki67 antibody (M00254-8).
Ki67 was detected in paraffin-embedded section of human esophageal squamous carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ki67 Antibody (M00254-8) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

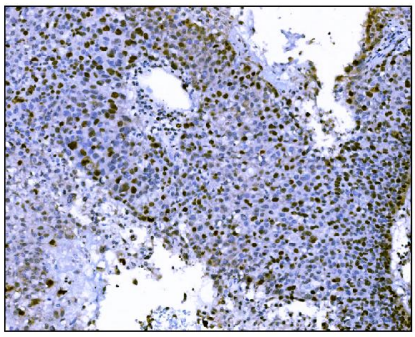
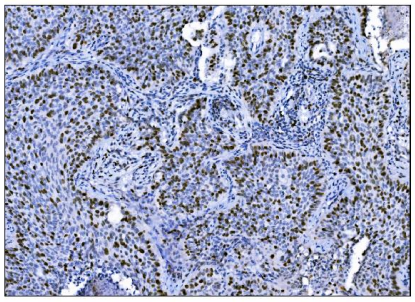


Figure 4. IHC analysis of Ki67 using anti-Ki67 antibody (M00254-8).
Ki67 was detected in paraffin-embedded section of human liver tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ki67 Antibody (M00254-8) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

Figure 5. IHC analysis of Ki67 using anti-Ki67 antibody (M00254-8).
Ki67 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with



10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ki67 Antibody (M00254-8) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

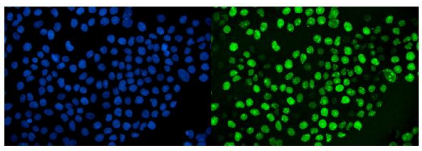


Figure 6. IF analysis of Ki67 using anti-Ki67 antibody (M00254-8).
Ki67 was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-Ki67 Antibody (M00254-8) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

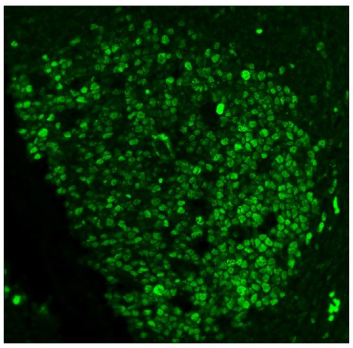


Figure 7. IF analysis of Ki67 using anti-Ki67 antibody (M00254-8).
Ki67 was detected in paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 µg/mL mouse anti-Ki67 Antibody (M00254-8) overnight at 4°C. Biotin conjugated goat anti-mouse IgG (BA1001) was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using DyLight®488 Conjugated Avidin (BA1128). The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

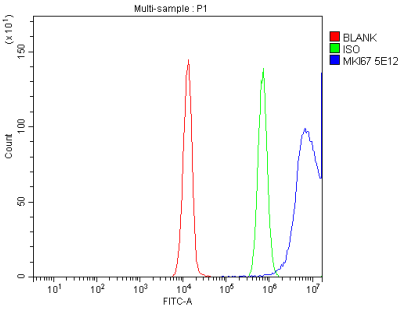


Figure 8. Flow Cytometry analysis of Jurkat cells using anti-Ki67 antibody (M00254-8).
Overlay histogram showing Jurkat cells stained with M00254-8 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Ki67 Antibody (M00254-8, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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