BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
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Anti-Ki67 Antibody (monoclonal, 5E12)

Catalog Number: M00254-8

About MKI67

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.

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Overview

Product Name	Anti-Ki67 Antibody (monoclonal, 5E12)
Reactive Species	Human
Description	Boster Bio Anti-Ki67 Antibody (monoclonal, 5E12) catalog # M00254-8. Tested in Flow Cytometry, IF, IHC, ICC applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, IHC, ICC
Clonality	Monoclonal 5E12
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Storage Instructions	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.
Host	Mouse
Uniprot ID	P46013

Technical Details

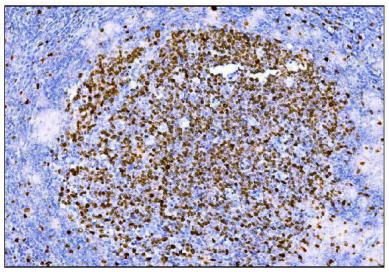
Immunogen	E. coli-derived human Ki67 recombinant protein (Position: K2860-I3256).
Recommended Detection Systems	Boster recommends HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.

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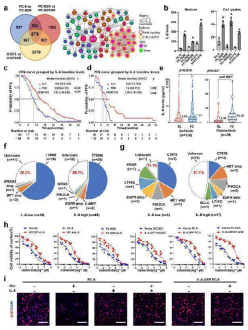
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2b
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human Immunocytochemistry/Immunofluorescence, 5ug/ml, Human Immunofluorescence, 5ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10^6 cells, Human Note: For flow cytometry (FCM), suitable for fixed cells only. Not suitable for FACS or surface flow cytometry using live cells.

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Anti-Ki67 Antibody (monoclonal, 5E12) (M00254-8) Images

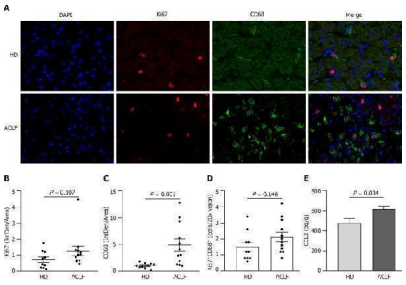


IHC analysis of Ki67 using anti-Ki67 antibody (M00254-8). Ki67 was detected in paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/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.

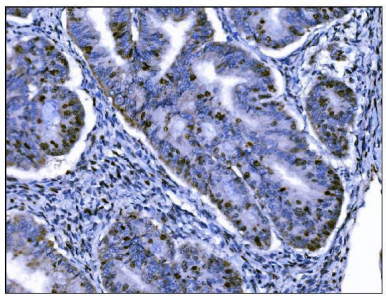


IL-6 was closely associated with osimertinib resistance. a High-throughput transcriptome sequencing in 3 paired cell strains sensitive/resistant to osimertinib, respectively, as indicated. Left: The Venn diagram of the commonly and exclusively differentially expressed genes (DEGs), and the overall overlapping site refers to the DEGs commonly found in all groups. Right: The protein–protein interaction network of 233 DEGs. Red and green nodes represent upregulated and downregulated genes after TKI resistance, respectively. The size of nodes refers to the degree of interaction with other genes. GR, gefitinib resistance; OR, osimertinib resistance. b ELISA assay of IL-6 levels in culture medium (pg/ml) or cell lysates (pg/100 ug protein) from paired osimertinib-sensitive and osimertinib-resistant cells, as indicated (n = 3 biologically independent experiments). Data are shown as mean ± SEM. *, $p < 0.01$ as compared to each parental cell strains. c and d Kaplan–Meier (KM) estimates of PFS in NSCLC patients treated with gefitinib, or osimertinib, respectively, according to high (greater than 7 mg/L) or low baseline IL-6 levels; e paired plasma IL-6 levels at baseline and disease progression of patients treated with gefitinib (n = 126) or osimertinib (n = 39). Lines indicate median and interquartile range of each group. P -value was calculated using a Mann–Whitney test. BL, baseline; PD, progression of disease. f gefitinib resistance mechanisms in low IL-6 group (n = 39) and high IL-6 group (n = 49), respectively. g Osimertinib resistance mechanisms in low IL-6 group (n = 9) and high IL-6 group (n = 17), respectively. h Cell viability CCK-8 assay for cells with different treatments as indicated. HCC827 cells, PC-9 cells, or PC-9GR cells were treated with IL-6 (20 ng/ml) together with increasing concentrations of osimertinib for 48 h. IL-6-GFP PC-9 cells, IL-6-GFP PC-9GR cells, and IL-6-GFP HCC827 cells were treated with indicated concentrations of osimertinib for 48 h. Data are shown as mean ± SEM (n = 3 biologically independent experiments). i Ki67 incorporation assay on PC-9 cells and IL-6-GFP PC-9 cells with different treatments as indicated. IL-6 (20 ng/ml) or osimertinib (1 uM) were added to the culture medium for 48 h. Cells were counterstained with DAPI. Scale bars: 100 um. Index in PubMed under a CC BY license. PMID: 35197546

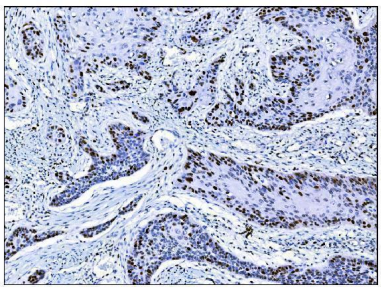
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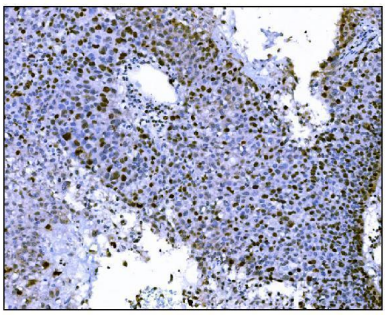
Macrophages were widely activated in the liver of patients with acute-on-chronic liver failure. A: Representative microscopic images of the double immunofluorescence staining of Ki67 and CD68 in the liver (400 fold). The red fluorescence signal represented Ki67; the green fluorescence signal represented CD68; and the blue fluorescence signal represented DAPI; B: Ki67 immunofluorescence intensity in the liver of healthy donors (n = 10) and patients with acute-on-chronic liver failure (n = 12); C: CD68 immunofluorescence intensity in the liver; D: The mean number of Ki67 + CD68 + -positive cells in five × 200 fields; E: Chemokine C-C motif ligand 2 levels in liver homogenates. Data with normal distribution were compared using unpaired student's t -test, and the Mann-Whitney test was used for non-normal data. ACLF: Acute-on-chronic liver failure; HD: Healthy donor. Index in PubMed under a CC BY license. PMID: 38516248



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 2ug/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.

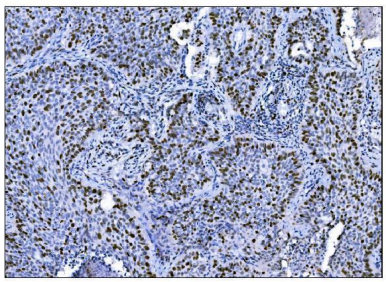


IHC analysis of Ki67 using anti-Ki67 antibody (M00254-8). Ki67 was detected in paraffin-embedded section of human esophageal squamous carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/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.

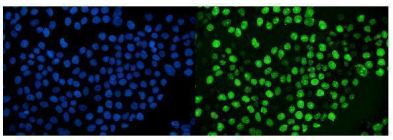


IHC analysis of Ki67 using anti-Ki67 antibody (M00254-8). Ki67 was detected in paraffin-embedded section of human liver tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/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.

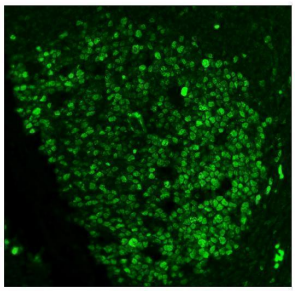
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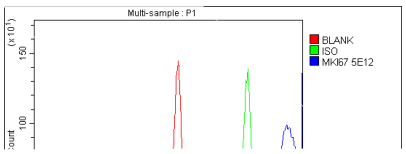
IHC analysis of Ki67 using anti-Ki67 antibody (M00254-8). Ki67 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/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.



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 5ug/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.



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 5ug/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.



Flow Cytometry analysis of Jurkat cells using anti-Ki67 antibody (M00254-8). Overlay histogram showing Jurkat cells stained with M00254-8 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Ki67 Antibody (M00254-8, 1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

1 Publications Citing This Product

1. PubMed ID: 31885629, Luo Z,Xu C,Li K,Xian B,Liu Y,Li K,Liu Y,Rong H,Tang M,Hu D,Yang S,Ye M,Zhong X,Ge J.Islet1 and Brn3 Expression Pattern

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Study in Human Retina and hiPSC-Derived Retinal Organoid.Stem Cells Int.2019 Dec 10;2019:8786396.doi:10.1155/2019/8786396.PMID:31885629;PMCID:PMC6925930.

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Anti-Ki67 Antibody (monoclonal, 5E12)

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