Product datasheet

Anti-Nuclear Matrix Protein p84 antibody [5E10] ab487

Customer reviews
★★★★★ 13 Abreviews  59 References  9 Images

Overview

Product name  Anti-Nuclear Matrix Protein p84 antibody [5E10]
Description  Mouse monoclonal [5E10] to Nuclear Matrix Protein p84
Host species  Mouse
Tested applications  Suitable for: WB, IP, ICC/IF, ICC, IHC-P, IHC-Fr, Flow Cyt
Species reactivity  Reacts with: Mouse, Human
Immunogen  Fusion protein containing amino acids 15-374 of human p84 expressed in E. coli.
General notes  This product was changed from ascites to tissue culture supernatant on 2nd Feb 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.

Properties

Form  Liquid
Storage instructions  Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer  pH: 7.40
  Constituent: PBS
Purity  Protein G purified
Clonality  Monoclonal
Clone number  5E10
Myeloma  NS1
Isotype  IgG2b
Light chain type  kappa

Applications

Our Abpromise guarantee covers the use of ab487 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
Function
Component of the THO subcomplex of the TREX complex. The TREX complex specifically associates with spliced mRNA and not with unspliced pre-mRNA. It is recruited to spliced mRNAs by a transcription-independent mechanism. Binds to mRNA upstream of the exon-junction complex (EJC) and is recruited in a splicing- and cap-dependent manner to a region near the 5' end of the mRNA where it functions in mRNA export. The recruitment occurs via an interaction between THOC4 and the cap-binding protein NCBP1. DDX39B functions as a bridge between THOC4 and the THO complex. The TREX complex is essential for the export of Kaposi's sarcoma-associated herpesvirus (KSHV) intronless mRNAs and infectious virus production. The recruitment of the TREX complex to the intronless viral mRNA occurs via an interaction between KSHV ORF57 protein and THOC4.

Regulates transcriptional elongation of a subset of genes. Participates in an apoptotic pathway which is characterized by activation of caspase-6, increases in the expression of BAK1 and BCL2L1 and activation of NF-kappa-B. This pathway does not require p53/TP53, nor does the presence of p53/TP53 affect the efficiency of cell killing. Activates a G2/M cell cycle checkpoint prior to the onset of apoptosis. Apoptosis is inhibited by association with RB1.

Tissue specificity
Ubiquitous. Expressed in various cancer cell lines. Expressed at very low levels in normal breast epithelial cells and highly expressed in breast tumors. Expression is strongly associated with an aggressive phenotype of breast tumors and expression correlates with tumor size and the metastatic state of the tumor progression.

Sequence similarities
Contains 1 death domain.

Domain
An intact death domain is needed for apoptosis.

Post-translational modifications
Expression is altered specifically during apoptosis and is accompanied by the appearance of novel forms with smaller apparent molecular mass.

Cellular localization

Form
Nuclear (Isoform 1) and Cytoplasmic (Isoform 1 and 2).

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>WB</td>
<td>🟣🟢🟢🟢🟢</td>
<td>Use a concentration of 0.3 - 2 µg/ml.</td>
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<tr>
<td>IP</td>
<td>🟣🟢🟢🟢🟢</td>
<td>1/100 - 1/1000.</td>
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<tr>
<td>ICC/IF</td>
<td>🟣🟢🟢🟢🟢</td>
<td>Use a concentration of 0.5 - 2 µg/ml.</td>
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<tr>
<td>ICC</td>
<td>🟣🟢🟢🟢🟢</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-P</td>
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<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-Fr</td>
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<td>Use at an assay dependent concentration.</td>
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<tr>
<td>Flow Cyt</td>
<td></td>
<td>Use 1µg for 10⁶ cells. ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.</td>
</tr>
</tbody>
</table>

Target

ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.
All lanes: Anti-Nuclear Matrix Protein p84 antibody [5E10] (ab487) at 1/500 dilution

**Lane 1**: HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

**Lane 2**: A431 (human epidermoid carcinoma cell line) whole cell lysate

**Lane 3**: HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 4**: HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

**Lane 5**: A-375 (human malignant melanoma cell line) whole cell lysate

Lysates/proteins at 30 µg per lane.

**Secondary**

All lanes: HRP-conjugated anti-mouse IgG

7.5% SDS-PAGE gel.

This image was generated using the ascites version of the product.

4% paraformaldehyde-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained for Nuclear Matrix Protein p84 (green) using ab487 at 1/500 dilution in ICC/IF.

Red: phalloidin, a cytoskeleton marker, at 1/200 dilution.

This image was generated using the ascites version of the product.
Western blot - Anti-Nuclear Matrix Protein p84 antibody [5E10] (ab487)

All lanes: Anti-Nuclear Matrix Protein p84 antibody [5E10] (ab487) at 1/1000 dilution

Lane 1: 0.1% DMSO treated Jurkat cell (whole cell lysate)
Lane 2: 0.5% DMSO treated Jurkat cell (whole cell lysate)
Lane 3: 1% DMSO treated Jurkat cell (whole cell lysate)
Lane 4: 0.1% SDS treated Jurkat cell (whole cell lysate)
Lane 5: 0.5% SDS treated Jurkat cell (whole cell lysate)
Lane 6: 1% SDS treated Jurkat cell (whole cell lysate)

Secondary
All lanes: HRP conjugated goat anti-mouse.

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 84 kDa
why is the actual band size different from the predicted?

Exposure time: 1 minute

SDS and DMSO were constituents of the lysis buffer. 0.1% SDS did not break down the nucleus entirely, however the higher concentrations did and p84 was detected in the lysate.

This image was generated using the ascites version of the product.

Immunocytochemistry/ Immunofluorescence - Anti-Nuclear Matrix Protein p84 antibody [5E10] (ab487)

ab487 staining Nuclear Matrix Protein p84 in Human stomach adenocarcinoma cell line (AGS) by Immunocytochemistry/Immunofluorescence. The cells were formaldehyde fixed, permeabilised in 0.025% Triton X-100, TBS and then blocked using 5% serum for 1 hour at 23°C. Samples were then incubated with primary antibody at 2µg/ml for 1 hour at 23°C. The secondary antibody used was a goat anti-mouse IgG conjugated to Alexa Fluor® 350 (blue) used undiluted, p84 shows nuclear localization.

This image was generated using the ascites version of the product.
ICC/IF image of ab487 stained Hepp cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab487, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

This image was generated using the ascites version of the product.

Overlay histogram showing HeLa cells stained with ab487 (red line). The cells were fixed with 100% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab487, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

This image was generated using the ascites version of the product.
Western blot - Anti-Nuclear Matrix Protein p84 antibody [5E10] (ab487)

Nuclear Matrix Protein p84 was immunoprecipitated from HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate with 3 µg ab487. Western blot was performed from the immunoprecipitate using ab487. Anti-Rabbit IgG was used as a secondary reagent.

Lane 1: HepG2 whole cell lysate 30 µg.
Lane 2: Control IP in HepG2 whole cell lysate with 3 µg of pre-immune mouse IgG.
Lane 3: ab487 IP in HepG2 whole cell lysate.

This image was generated using the ascites version of the product.

All lanes : Anti-Nuclear Matrix Protein p84 antibody [5E10] (ab487) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) nuclear lysate

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : HRP-conjugated anti-mouse IgG

7.5% SDS-PAGE gel.
This image was generated using the ascites version of the product.
Acetone-fixed frozen section of human stomach tissue stained for Nuclear Matrix Protein p84 using ab487 at 2 µg/ml in immunohistochemical analysis.

This image was generated using the ascites version of the product.

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