Product datasheet

Anti-JNK1+JNK2 (phospho T183 + Y185) antibody
ab4821

Overview

Product name
Anti-JNK1+JNK2 (phospho T183 + Y185) antibody

Description
Rabbit polyclonal to JNK1+JNK2 (phospho T183 + Y185)

Host species
Rabbit

Specificity
Phosphorylation site-specific antibody selective for the dually phosphorylated form of the c-Jun N-terminal Kinase (JNK)/Stress-Activated Protein Kinase (SAPK) enzymes containing a phosphate on threonine 183 and tyrosine 185 (human JNK 1 + 2). The antibody has been shown to recognize the endogenous, active forms of JNK 1 + 2 in a variety of cell types following treatment by a broad range of extracellular stimuli [e.g. including 293 cells (human embryonic kidney; +/- ultraviolet light) and PC12 cells (rat pheochromocytoma; +/- sorbitol)]. The region of JNK1 and JNK2 surrounding T183 + Y185 has a high degree of similarity to the corresponding regions in JNK3 and thus may cross react with this protein if phosphorylated on the corresponding residues.

Tested applications
Suitable for: ICC/IF, IHC-P, IHC-Fr, WB

Species reactivity
Reacts with: Mouse, Human
Predicted to work with: a wide range of other species

Immunogen
Synthetic peptide (Human) derived from the region of JNK 1 + 2 that contains threonine 183 and tyrosine 185, based on the human sequence. This region is conserved among many species including human, rat, mouse, chick, nematode (Caenorhabditis elegans), and fly (Drosophila melanogaster).

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: 7.30
Preservative: 0.05% Sodium azide
 Constituents: PBS, 50% Glycerol, 0.1% BSA

Purity
Immunogen affinity purified

BSA is IgG and protease free

Purification notes
Purified from rabbit serum by sequential epitope specific chromatography. The antibody has been
negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated JNK enzymes. The final product is generated by affinity chromatography using a JNK-derived peptide that is phosphorylated at threonine 183 and tyrosine 185, within the activation loop. Note: It is the dually phosphorylated form of these enzymes that has full enzymatic activity.

Clonality
Polyclonal

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of **ab4821** in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>★★★★☆</td>
<td>1/1 - 1/100. 1/100.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★☆</td>
<td>Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td>★★★★☆</td>
<td>1/1000. Predicted molecular weight: 49, 55 kDa. Band at ~49 kDa represents Jnk1, while the band at ~55 kDa represents Jnk2</td>
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Target

Function
Responds to activation by environmental stress and pro-inflammatory cytokines by phosphorylating a number of transcription factors, primarily components of AP-1 such as JUN, JDP2 and ATF2 and thus regulates AP-1 transcriptional activity. In T-cells, JNK1 and JNK2 are required for polarized differentiation of T-helper cells into Th1 cells (By similarity). Phosphorylates heat shock factor protein 4 (HSF4). JNK1 isoforms display different binding patterns: beta-1 preferentially binds to c-Jun, whereas alpha-1, alpha-2, and beta-2 have a similar low level of binding to both c-Jun or ATF2. However, there is no correlation between binding and phosphorylation, which is achieved at about the same efficiency by all isoforms.

Sequence similarities
Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily. Contains 1 protein kinase domain.

Domain
The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the MAP kinases.

Post-translational modifications
Dually phosphorylated on Thr-183 and Tyr-185, which activates the enzyme.

Images
MEF1 cells were incubated at 37°C for 48h with vehicle control (0 µM) and 5 µM of glibenclamide (ab120267) in DMSO. Increased expression of of JNK1+JNK2 (phospho T183 + Y185) (ab4821) correlates with an increase in glibenclamide concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 10µg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 3% milk before being incubated with ab4821 at 1/1000 dilution and ab85139 at 1 µg /ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (ab97051) at 1/10000 dilution and visualised using ECL development solution.

ab4821 staining JNK1 + JNK2 (phospho T183 + Y185) in A549 cells (green, panel a) by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% paraformaldehyde, permeabilized with 0.25% Triton X-100 and blocked with 5% BSA for 1 hour at room temperature. Samples were incubated with primary antibody (2ug/ml in 1% BSA) for 3 hours at room temperature. An Alexa Fluor® 488-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody (1/400). Nuclei stained with DAPI (blue, panel b), F-actin stained with Alexa Fluor® 594 Phalloidin (red, panel b) and merged images (panel d).
To demonstrate the phosphorylation of JNK 1 & 2 in a cell based assay, 293 cells were treated with ultraviolet irradiation (UV). Proteins from cell extracts were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to nitrocellulose. Membranes were incubated with either 1 µg/mL ab4821 or 1 µg/mL anti-JNK1 pan. After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG alkaline phosphatase and bands were detected using the Tropix WesternStar detection method.

To demonstrate the phosphorylation of JNK 1 & 2 in a cell based assay, 293 cells were treated with ultraviolet irradiation (UV). Proteins from cell extracts were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to nitrocellulose. Membranes were incubated with either 1 µg/mL ab4821 or 1 µg/mL anti-JNK1 pan. After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG alkaline phosphatase and bands were detected using the Tropix WesternStar detection method.

MCF7 cells were incubated at 37°C for 4h with vehicle control (0 µM) and different concentrations of cryptotanshinone (ab120666). Increased expression of JNK1+JNK2 (phospho T183 + Y185) in MCF7 cells correlates with an increase in cryptotanshinone concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 10µg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with ab4821 at 1/1000 dilution and ab8227 at 1 µg/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (ab97051) at 1/10000 dilution and visualised using ECL development solution.

ab4821 staining JNK1+JNK2 (phospho T183 + Y185) in E12.5 mouse heart tissue section by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue underwent cold 1% paraformaldehyde fixation before heat mediated antigen retrieval with Tris-EDTA pH9 and then blocking with 2.25% horse serum was performed for 20 minutes at RT. The primary antibody was diluted 1/400 and incubated with sample for 12 hours at 4°C. A Biotin conjugated horse polyclonal to rabbit IgG was used at dilution at 1/133 as secondary antibody.
Immunocytochemistry/ Immunofluorescence - Anti-JNK1+JNK2 (phospho T183 + Y185) antibody (ab4821)

This image is courtesy of an Abreview submitted by Mr George Chennell

ab4821 staining JNK1+JNK2 (phospho T183 + Y185) in human foreskin fibroblasts by ICC/IF. The cells were fixed in cytoskeletal fixative, permeabilized in 0.5% Triton X-100 and blocked in 2% dilution buffer (2%BSA + 0.1% Triton X-100) for 1 hour at 25°C. The primary antibody was diluted, 1/100 and incubated with sample for 12 hours. An Alexa Fluor® 594 conjugated goat polyclonal to rabbit IgG, diluted 1/250 was used as secondary.

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