Product datasheet

Anti-c-Jun (phospho S63) antibody [Y172] ab32385

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Overview

Product name  Anti-c-Jun (phospho S63) antibody [Y172]
Description  Rabbit monoclonal [Y172] to c-Jun (phospho S63)
Host species  Rabbit
Specificity  The antibody only detects c-Jun phosphorylated on Serine 63 when tested in WB and ICC using specific phospho-treatments. However, in DotBlot and ELISA assays we detected some cross-reactivity with the non-phospho peptide as well. Please refer to the images on the datasheet. The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse.

Tested applications

Suitable for: WB, IHC-P, ICC/IF, Dot blot, ELISA
Unsuitable for: Flow Cyt

Species reactivity

Reacts with: Mouse, Human
Predicted to work with: Rat, Cow

Immunogen  Synthetic peptide within Human c-Jun aa 50-150 (phospho S63). The exact sequence is proprietary.
Database link: P05412

Positive control  WB: UV or Anisomycin treated NIH/3T3 or HeLa cell lysate. IHC-P: Human breast carcinoma tissue. ICC/IF: A431 cells.

General notes  A trial size is available to purchase for this antibody.

Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

Properties

Form  Liquid
Applications

Our Abpromise guarantee covers the use of ab32385 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>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td>1/1000 - 1/10000. Detects a band of approximately 42 kDa (predicted molecular weight: 36 kDa).</td>
<td></td>
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<tr>
<td>IHC-P</td>
<td>1/250. See IHC antigen retrieval protocols. For unpurified use at 1/50 - 1/100</td>
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<tr>
<td>ICC/IF</td>
<td>1/100 - 1/200.</td>
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<tr>
<td>Dot blot</td>
<td>1/1000.</td>
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<tr>
<td>ELISA</td>
<td>Use at an assay dependent concentration.</td>
<td></td>
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</table>

Application notes

Is unsuitable for Flow Cyt.

Target

Function


Sequence similarities

Belongs to the bZIP family. Jun subfamily. Contains 1 bZIP (basic-leucine zipper) domain.

Post-translational modifications

Ubiquitinated by the SCF(FBXW7), leading to its degradation. Ubiquitination takes place following phosphorylation, that promotes interaction with FBXW7. Phosphorylated by CaMK4 and PRKDC; phosphorylation enhances the transcriptional activity. Phosphorylated by HIPK3. Phosphorylated by DYRK2 at Ser-243; this primes the protein for subsequent phosphorylation by GSK3B at Thr-239. Phosphorylated at Thr-239, Ser-243 and Ser-249 by GSK3B; phosphorylation reduces its ability to bind DNA. Phosphorylated by PAK2 at Thr-2, Thr-8, Thr-89, Thr-93 and Thr-286 thereby promoting JUN-mediated cell proliferation and transformation. Phosphorylated by PLK3 following hypoxia or UV irradiation, leading to increase
DNA-binding activity.
Acetylated at Lys-271 by EP300.

**Cellular localization**
Nucleus.

**Images**

**Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast tissue sections labeling c-Jun with Purified ab32385 at 1:250 dilution (0.46 µg/ml).** Heat mediated antigen retrieval was performed using ab93884 (Tris/EDTA buffer, pH 9.0) ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

**Western blot - Anti-c-Jun (phospho S63) antibody [Y172] (ab32385)**

All lanes: Anti-c-Jun (phospho S63) antibody [Y172] (ab32385) at 0.1 µg/ml (purified)

Lane 1: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

Lane 2: NIH/3T3 (Mouse embryonic fibroblast) treated with 250 ng/ml anisomycin for 30 minutes whole cell lysates

Lane 3: NIH/3T3 (Mouse embryonic fibroblast) treated with 250 ng/ml anisomycin for 30 minutes whole cell lysates. Then the membrane was incubated with phosphatase

Lysates/proteins at 15 µg per lane.

**Secondary**

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size:** 36 kDa

Blocking and diluting buffer: 5% NFDM/TBST.
**Western blot - Anti-c-Jun (phospho S63) antibody [Y172] (ab32385)**

**All lanes**: Anti-c-Jun (phospho S63) antibody [Y172] (ab32385) at 0.1 µg/ml (purified)

**Lane 1**: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

**Lane 2**: HeLa (Human cervix adenocarcinoma epithelial cell) treated with 1 ug/ml anisomycin for 15 minutes whole cell lysates

**Lane 3**: HeLa (Human cervix adenocarcinoma epithelial cell) treated with 1 ug/ml anisomycin for 15 minutes whole cell lysates 15ug. Then the membrane was incubated with phosphatase.

Lysates/proteins at 15 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size**: 36 kDa

Blocking and diluting buffer: 5% NFDM/TBST.

**Lane 1**: Anti-c-Jun (phospho S63) antibody [Y172] (ab32385) at 1/1000 dilution (Unpurified)

**Lanes 2-3**: Human HRPT2 peptide (ab23385) at 1/1000 dilution (Unpurified)

**Lane 1**: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates with NFDM/TBST

**Lane 2**: HeLa (Human cervix adenocarcinoma epithelial cell) treated with 1ug/mL anisomycin for 15 minutes whole cell lysates with NFDM/TBST

**Lane 3**: HeLa (Human cervix adenocarcinoma epithelial cell) treated with 1ug/ml anisomycin for 15 minutes whole cell lysates. Then the membrane was incubated with phosphatase with NFDM/TBST

Lysates/proteins at 15 µg per lane.

Blocking peptides at 5 % per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A431 (Human epidermoid carcinoma cell line) cells labeling c-Jun (phospho S63) with ab32385 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing the expression was increased after treatment with anisomycin (1 µg/ml for 15 minutes), then decreased after treatment with the Lambda Protein Phosphatase treatment for 2 hours. The nuclear counter stain is DAPI (blue). Counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at a 1/200 dilution (red).

Antigen pS63:c-Jun (phospho S63); NP:c-Jun non-phospho.
Antigen concentration 0.01~1 ng/ml.
Primary antibody concentration range 0~1000 ng/ml.
Secondary antibody is an Alkaline Phosphatase-conjugated Goat Anti-Rabbit IgG(H+L) used at a 1:2500 dilution.

Unpurified ab32385 used at a 1:1000 dilution.
Secondary antibody is Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) used at a 1:100,000 dilution.
Blocking/Diluting buffer and concentration: 5% NFDM/TBST.
Lane 1: c-Jun (pS63) phospho peptide.
Lane 2: c-Jun non-phospho peptide.
Exposure time 3 minutes.
Western blot - Anti-c-Jun (phospho S63) antibody [Y172] (ab32385)

All lanes: Anti-c-Jun (phospho S63) antibody [Y172] (ab32385) at 1/10000 dilution (Unpurified)

Lanes 1 & 3: Untreated NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate
Lane 2: NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate treated with ultraviolet light
Lane 4: NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate treated with 25 µg/ml Anisomycin for 15 minutes at 37°C

Predicted band size: 36 kDa
Observed band size: 42 kDa
why is the actual band size different from the predicted?

Paraffin-embedded human breast carcinoma tissue stained for c-Jun (phospho S63) with unpurified ab32385 at a 1/50 dilution in immunohistochemical analysis.

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