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

Description: Rabbit monoclonal [Y172] to c-Jun (phospho S63)

Host species: Rabbit

Specificity: Anti-c-Jun (phospho S63) antibody [Y172] (ab32385) 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 whole cell lysate (ab150035). IHC-P: Human breast carcinoma tissue. ICC/IF: A431 cells, NIH/3T3 cells.

General notes: This product is a recombinant monoclonal antibody, which offers several advantages including:
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.
Form     Liquid
Storage buffer  pH: 7.20
Preservative: 0.01% Sodium azide
 Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity  Protein A purified
Clonality  Monoclonal
Clone number  Y172
Isotype  IgG

Applications

The Abpromise guarantee  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>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐ (2)</td>
<td>1/1000 - 1/10000. Detects a band of approximately 42 kDa (predicted molecular weight: 36 kDa).</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/50 - 1/100 The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>1/100 - 1/200.</td>
</tr>
<tr>
<td>Dot blot</td>
<td></td>
<td>1/1000.</td>
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<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

Application notes  Is unsuitable for Flow Cyt.

Target

Function  Transcription factor that recognizes and binds to the enhancer heptamer motif 5'-TGA[CG]TCA-3'. Promotes activity of NR5A1 when phosphorylated by HIPK3 leading to increased steroidogenic gene expression upon cAMP signaling pathway stimulation. Involved in activated KRAS-mediated transcriptional activation of USP28 in colorectal cancer (CRC) cells (PubMed:24623306). Binds to the USP28 promoter in colorectal cancer (CRC) cells (PubMed:24623306).

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**

Immunocytochemistry confocal image of 4% paraformaldehyde-fixed 0.1% Triton X-100 permeabilized anisomycin-treated NIH/3T3 cell line (mouse embryonic fibroblast), staining nuclear c-Jun with ab32385 at 1:500 dilution and ab150077 AlexaFluor®488 Goat anti-Rabbit secondary at 1:1000 dilution. The counterstain was ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1:200 dilution, and the nuclear counterstain was DAPI (blue).

The NIH/3T3 cells were treated with 250 ng/ml Anisomycin for 30 minutes and then the signal decreased after phosphatase treatment at 37°C for 2 hours.

**Western blot**

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.

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 ab93684 (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.

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).
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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32385).
Lane 1: Anti-c-Jun (phospho S63) antibody [Y172] (ab32385) at 1/1000 dilution (Unpurified)

Lanes 2-3: Human HRPT2/Parafibromin 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 1µg/mL anisomycin for 15 minutes whole cell lysates with NFDM/TBST

Lane 3: HeLa (Human cervix adenocarcinoma epithelial cell) treated with 1µg/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

Predicted band size: 36 kDa
ELISA - Anti-c-Jun (phospho S63) antibody [Y172] (ab32385)

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.

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

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: Human c-Jun (pS63) phospho peptide.
Lane 2: Human c-Jun non-phospho peptide.
Exposure time 3 minutes.
All lanes: Anti-c-Jun (phospho S63) antibody [Y172] (ab32385) at 1/10000 dilution (Unpurified)

Lanes 1 & 3: Untreated NIH/3T3 (Mouse embyro fibroblast cell line) cell lysate
Lane 2: NIH/3T3 (Mouse embyro fibroblast cell line) cell lysate treated with ultraviolet light
Lane 4: NIH/3T3 (Mouse embyro 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

Paraffin-embedded human breast carcinoma tissue stained for c-Jun (phospho S63) with unpurified ab32385 at a 1/50 dilution in immunohistochemical analysis.
Anti-c-Jun (phospho S63) antibody [Y172] (ab32385)

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