# abcam

### **Product datasheet**

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

重组 RabMAb

★★★★★ 4 Abreviews 78 References 11 图像

Anti-c-Jun (phospho S63) <b>抗体</b> [Y172]		
免单克隆抗体[Y172] to c-Jun (phospho S63)		
Rabbit		
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.		
适用于: WB, IHC-P, ICC/IF, Dot blot, ELISA <b>不适用于:</b> Flow Cyt		
<b>与反</b> 应: Mouse, Human		
预测 <b>可用于:</b> Rat, Cow 🔺		
Synthetic peptide within Human c-Jun aa 50-150 (phospho S63). The exact sequence is proprietary. Database link: <b>P05412</b>		
WB: UV or Anisomycin treated NIH/3T3 or HeLa whole cell lysate ( <u>ab150035</u> ). IHC-P: Human breast carcinoma tissue. ICC/IF: A431 cells, NIH/3T3 cells.		
<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>		

形式	Liquid
存 <b>放</b> 说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯 <b>度</b>	Protein A purified
克隆	单 <b>克隆</b>
<b>克隆</b> 编号	Y172
同种型	lgG

应用

#### The Abpromise guarantee Abpromise™承诺保证使用ab32385于以下的经测试应用

#### "应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★ ★ ★ ★ ★ <u>(2)</u>	1/1000 - 1/10000. Detects a band of approximately 42 kDa (predicted molecular weight: 36 kDa).
IHC-P		<ul> <li>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.</li> <li>For unpurified use at 1/50 - 1/100</li> <li>The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse.</li> </ul>
ICC/IF		1/100 - 1/200.
Dot blot		1/1000.
ELISA		Use at an assay dependent concentration.

应用说明

Is unsuitable for Flow Cyt.

靶标	
功能	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).
序列相似性	Belongs to the bZIP family. Jun subfamily. Contains 1 bZIP (basic-leucine zipper) domain.
<b>翻</b> 译后修饰	Ubiquitinated by the SCF(FBXW7), leading to its degradation. Ubiquitination takes place following phosphorylation, that promotes interaction with FBXW7.

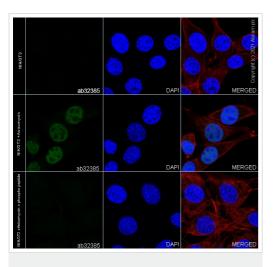
Phosphorylated by CaMK4 and PRKDC; phosphorylation enhances the transcriptional activity. Phosphorylated by HIPK3. Phosphorylated by DYRK2 at Ser-243; this primes the protein for subsequent phosphorylation by GSK3B at Thr-239. Phosphorylated at Thr-239, Ser-243 and Ser-249 by GSK3B; phosphorylation reduces 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.

细胞定位

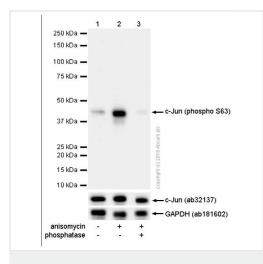
Nucleus.

#### 图片



Immunocytochemistry/ Immunofluorescence - Antic-Jun (phospho S63) antibody [Y172] (ab32385) Immunocytochemistry confocal image of 4% paraformaldehydefixed 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 <u>ab150077</u> AlexaFluor®488 Goat anti-Rabbit secondary at 1:1000 dilution. The counterstain was <u>ab195889</u> 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 - 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

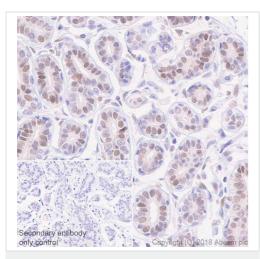
Lysates/proteins at 15 µg per lane.

#### Secondary

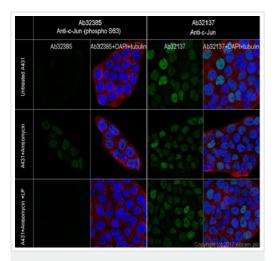
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 36 kDa

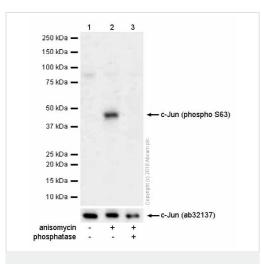
Blocking and diluting buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Jun (phospho S63) antibody [Y172] (ab32385) 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 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.Hematoxylinwas used as a counterstain



Immunocytochemistry/ Immunofluorescence - Antic-Jun (phospho S63) antibody [Y172] (ab32385) 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<sup>®</sup> 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 31**I** for 2 hours. The nuclear counter stain is DAPI (blue). Counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 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.

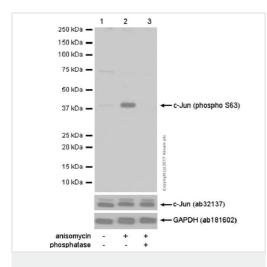
#### Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) 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).



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

Lanes 2-3 : Human HRPT2/Parafibromin peptide (<u>ab23385</u>) 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 1 ug/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

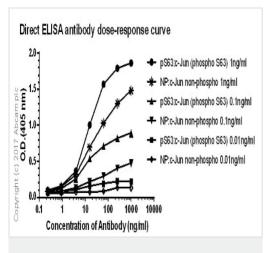
Lysates/proteins at 15 µg per lane.

Blocking peptides at 5 % per lane.

#### Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 36 kDa

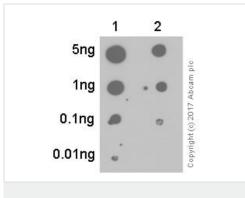


Antigen pS63:c-Jun (phospho S63); NP:c-Jun non-phospho. Antigen concentration 0.01~1 ng/ml.

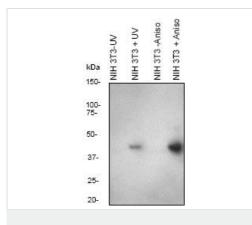
Primary antibody concentration range 0~1000 ng/ml.

Secondary antibody is an Alkaline Phosphatase-conjugated Goat Anti-Rabbit IgG(H+L) used at a 1:2500 dilution.

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



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.



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

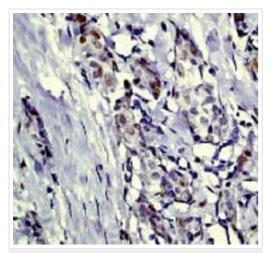
**All lanes :** Anti-c-Jun (phospho S63) antibody [Y172] (ab32385) at 1/10000 dilution (Unpurified)

Lanes 1 & 3 : Untreated NIH/3T3 (Mouse embyro fibroblast cell line) cell lysate

Lane 2 : NIH/3T3 (Mouse embyro fibroblast cell line) cell lysate treated with ultraviolet light

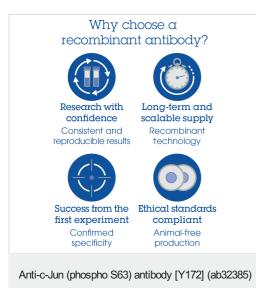
Lane 4 : NIH/3T3 (Mouse embyro fibroblast cell line) cell lysate treated with 25  $\mu$ g/ml Anisomycin for 15 minutes at 37°C

Predicted band size: 36 kDa Observed band size: 42 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Jun (phospho S63) antibody [Y172] (ab32385)

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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