abcam

Product datasheet

Anti-c-Jun antibody [EP693Y] ab40766

KO VALIDATED Recombinant RabMAb

***** 1 Abreviews 28 References 11 Images

Overview

iti-c-Jun antibody [EP693Y] Ibbit monoclonal [EP693Y] to c-Jun Ibbit
lbbit
3S only lot tested.
itable for: Flow Cyt (Intra), ICC/IF, WB, IHC-P, IP
eacts with: Mouse, Rat, Human
nthetic peptide. This information is proprietary to Abcam and/or its suppliers.
B: HEK-293, MOJ/3T3 and PC-12 cell lysates;. IHC-P: Rat liver, mouse cerebrum and human rvix carcinoma tissues. ICC/IF: NIH/3T3 and HeLa cells. ICC/IF KO: HEK293 cells (HEK293- IN KO cells used as a negative cell line). Flow Cyt (intra): HEK-293 cells IP: NIH/3T3 cell lysate
is product is a recombinant monoclonal antibody, which offers several advantages including: ligh batch-to-batch consistency and reproducibility nproved sensitivity and specificity ong-term security of supply unimal-free production or more information <u>see here</u> . Ir RabMAb [®] technology is a patented hybridoma-based technology for making rabbit phoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u> .

Properties	
Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP693Y
Isotype	lgG

Applications

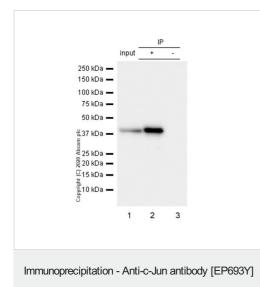
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab40766 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

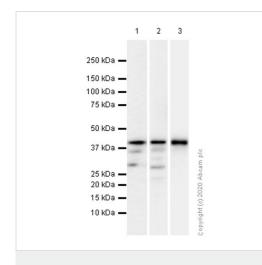
Application	Abreviews	Notes
Flow Cyt (Intra)		1/20.
ICC/IF		1/50. Signal can be observed in cells fixed with either methanol or paraformaldehyde.
WB		1/1000 - 1/5000. Detects a band of approximately 39 kDa (predicted molecular weight: 39 kDa).
IHC-P	★ ★ ★ ★ ★ (1)	1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/20.

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







Western blot - Anti-c-Jun antibody [EP693Y] (ab40766) Purified ab40766 at 1/20 dilution (1µg) immunoprecipitating c-Jun in NIH/3T3 whole cell lysate. Lane 1 (input): NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate 10µg

Lane 2 (+): ab40766 + NIH/3T3 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab40766 in NIH/3T3 whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (ab131366) (1/1000

dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 39 kDa

All lanes : Anti-c-Jun antibody [EP693Y] (ab40766) at 1/1000 dilution (Purified)

Lane 1 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate

Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate

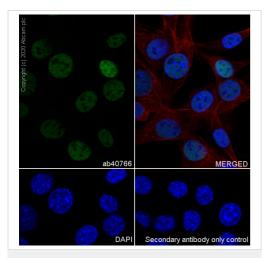
Lysates/proteins at 15 µg per lane.

Secondary

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

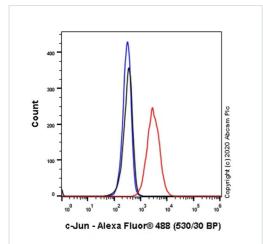
Predicted band size: 39 kDa Observed band size: 39 kDa

Blocking Buffer and concentration: 5% NFDM/TBST

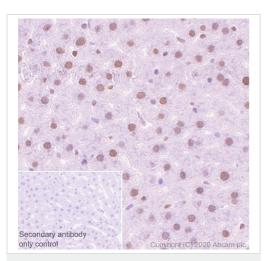


Immunocytochemistry/ Immunofluorescence - Antic-Jun antibody [EP693Y] (ab40766)

Immunocytochemistry analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labeling c-Jun with Purified ab40766 at 1:50 dilution (4.06 ?g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) 1:200 (2.5 μ g/ml). Goat anti rabbit lgG (Alexa Fluor[®] 488, <u>ab150077</u>) was used as the secondary antibody at 1:1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

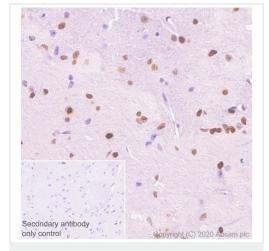


Flow Cytometry (Intracellular) - Anti-c-Jun antibody [EP693Y] (ab40766) Intracellular Flow Cytometry analysis of HEK-293 (Human embryonic kidney epithelial cell) cells labeling c-Jun with Purified ab40766 at 1/20 dilution (10µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr[®] 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



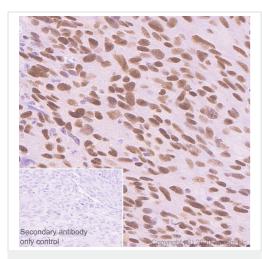
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat liver tissue sections labeling c-Jun with Purified ab40766 at 1:500 dilution (0.41 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Jun antibody [EP693Y] (ab40766)



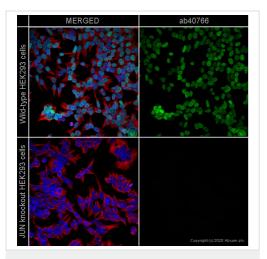
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Jun antibody [EP693Y] (ab40766)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse cerebrum tissue sections labeling c-Jun with Purified ab40766 at 1:500 dilution (0.41 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Jun antibody [EP693Y] (ab40766)

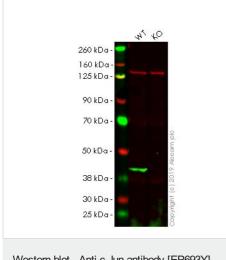
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue sections labeling c-Jun with Purified ab40766 at 1:500 dilution (0.41 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunocytochemistry/ Immunofluorescence - Antic-Jun antibody [EP693Y] (ab40766)

ab40766 staining c-Jun in wild-type HEK293 cells (top panel) and c-Jun knockout HEK293 cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab40766 at 1/250 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit lgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse lgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a high-content analysis system (Perkin Elmer, Operetta CLS™).



Western blot - Anti-c-Jun antibody [EP693Y] (ab40766) **All lanes :** Anti-c-Jun antibody [EP693Y] (ab40766) at 1/1000 dilution

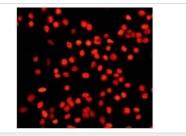
Lane 1 : Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 2 : Jun knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lysates/proteins at 40 µg per lane.

Predicted band size: 39 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab40766 observed at 35 kDa. Red - loading control, <u>ab18058</u>, observed at 130 kDa.

ab40766 was shown to specifically react with Jun in wild-type HEK-293 cells as signal was lost in Jun knockout cells. Wild-type and Jun knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab40766 and <u>ab18058</u> (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Antic-Jun antibody [EP693Y] (ab40766)



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8

Immunofluorescent staining of HeLa cells ab40766 at 1/100 dilution