# abcam

# Product datasheet

# Anti-c-Jun antibody [EP693Y] ab40766



ייבער RabMAb

★★★★★ 1 Abreviews 28 References 画像数 11

### 製品の概要

製品名 Anti-c-Jun antibody [EP693Y]

製品の詳細 Rabbit monoclonal [EP693Y] to c-Jun

由来種 Rabbit

特異性 PBS only lot tested.

アプリケーション 適用あり: Flow Cyt (Intra), ICC/IF, WB, IHC-P, IP

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: HEK-293, MOJ/3T3 and PC-12 cell lysates;. IHC-P: Rat liver, mouse cerebrum and human

> cervix carcinoma tissues. ICC/IF: NIH/3T3 and HeLa cells. ICC/IF KO: HEK293 cells (HEK293-JUN KO cells used as a negative cell line). Flow Cyt (intra): HEK-293 cells IP: NIH/3T3 cell lysate

特記事項 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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

#### 製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA

精製度 Protein A purified

**ポリ/モノ** モノクローナル

**クローン名** EP693Y

アイソタイプ lgG

#### アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab40766の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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	**** <u>(1)</u>	1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/20.

## ターゲット情報

機能 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).

配列類似性 Belongs to the bZIP family. Jun subfamily.

Contains 1 bZIP (basic-leucine zipper) domain.

翻訳後修飾 Ubiquitinated by the SCF(FBXW7), leading to its degradation. Ubiquitination takes place

following phosphorylation, that promotes interaction with FBXW7.

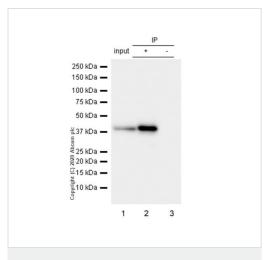
Phosphorylated by CaMK4 and PRKDC; phosphorylation enhances the transcriptional activity. Phosphorylated by HIPK3. Phosphorylated by DYRK2 at Ser-243; this primes the protein for subsequent phosphorylation by GSK3B at Thr-239. Phosphorylated at Thr-239, Ser-243 and Ser-249 by GSK3B; phosphorylation reduces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

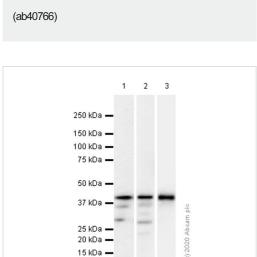
DNA-binding activity.

Acetylated at Lys-271 by EP300.

細胞内局在 Nucleus.



Immunoprecipitation - Anti-c-Jun antibody [EP693Y]



Western blot - Anti-c-Jun antibody [EP693Y] (ab40766)

10 kDa 🗕

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 lgG (ab172730) 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

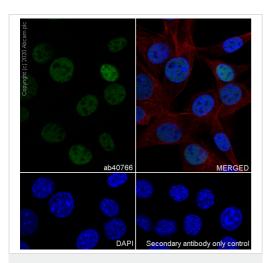
Lysates/proteins at 15 µg per lane.

#### **Secondary**

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

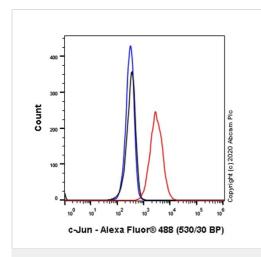
Predicted band size: 39 kDa Observed band size: 39 kDa

Blocking Buffer and concentration: 5% NFDM/TBST



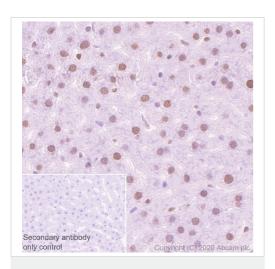
Immunocytochemistry/ Immunofluorescence - Antic-Jun antibody [EP693Y] (ab40766)

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  $\mu$ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) was used as the secondary antibody at 1:1000 (2  $\mu$ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



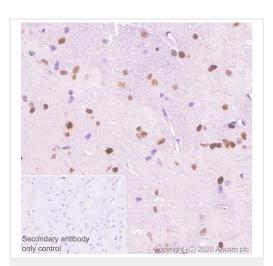
Flow Cytometry (Intracellular) - Anti-c-Jun antibody [EP693Y] (ab40766)

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).



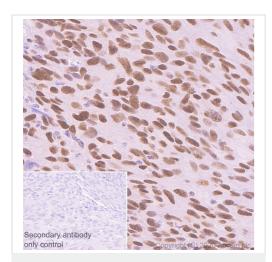
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Jun antibody [EP693Y] (ab40766)

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.



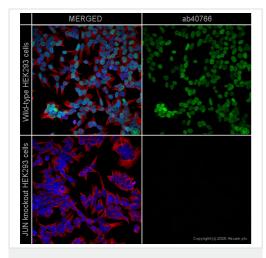
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Jun antibody [EP693Y] (ab40766)

Immunohistochemistry (Formalin/PFA-fixed 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)

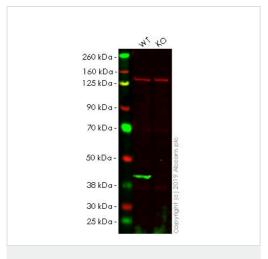
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue sections labeling c-Jun with Purified ab40766 at 1:500 dilution (0.41 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection 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  $^{\text{TM}}$ ).



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

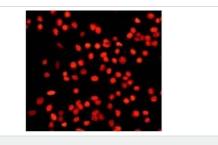
**Lane 2**: Jun knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lysates/proteins at 40 µg per lane.

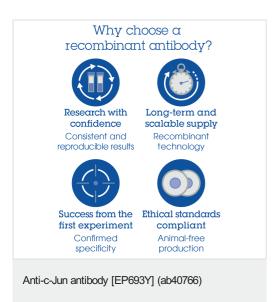
Predicted band size: 39 kDa

**Lanes 1 - 2:** Merged signal (red and green). Green - ab40766 observed at 35 kDa. Red - loading control, **ab18058**, 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 <a href="mailto:ab18058">ab18058</a> (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 <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Antic-Jun antibody [EP693Y] (ab40766) Immunofluorescent staining of HeLa cells ab40766 at 1/100 dilution



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

#### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- · We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.co.jp/abpromise">https://www.abcam.co.jp/abpromise</a> or contact our technical team.

#### Terms and conditions

· Guarantee only valid for products bought direct from Abcam or one of our authorized distributors