abcam

Product datasheet

Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] ab179461

Recombinant RabMAb

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Overview

Product name Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211]

Description Rabbit monoclonal [EPR16797-211] to JNK1 + JNK2 + JNK3

Host species Rabbit

Tested applications Suitable for: WB, IP, ICC/IF, Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat, Chicken, Cow, Dog, Human, Zebrafish, African green monkey,

Xenopus tropicalis

Predicted to work with: Monkey

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human JNK1, JNK2 and JNK3 full length recombinant proteins; K562, HeLa, Jurkat, Neuro-

2a, UMNSAH/DF-1, MDCK, MDBK and COS-1 whole cell lysates; Zebrafish and X. tropicalis lysates. Mouse brain, Rat brain, Rat heart, RAW 264.7, PC-12 and NIH/3T3 lysates. ICC/IF: HeLa

cells. IP: Jurkat whole cell extract. Flow Cyt (intra): HeLa cells

General notesThis 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**.

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 long

term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

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Purity Protein A purified

Clonality Monoclonal

Clone number EPR16797-211

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab179461 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
WB	★★★★☆ (4)	1/1000. Detects a band of approximately 54, 46 kDa (predicted molecular weight: 48 kDa).
IP		1/50.
ICC/IF	★★★★ <u>(3)</u>	1/250.
Flow Cyt (Intra)		1/180. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

Target

Function

Serine/threonine-protein kinase involved in various processes such as cell proliferation, differentiation, migration, transformation and programmed cell death. Extracellular stimuli such as proinflammatory cytokines or physical stress stimulate the stress-activated protein kinase/c-Jun N-terminal kinase (SAP/JNK) signaling pathway. In this cascade, two dual specificity kinases MAP2K4/MKK4 and MAP2K7/MKK7 phosphorylate and activate MAPK8/JNK1. In turn, MAPK8/JNK1 phosphorylates a number of transcription factors, primarily components of AP-1 such as JUN, JDP2 and ATF2 and thus regulates AP-1 transcriptional activity. Phosphorylates the replication licensing factor CDT1, inhibiting the interaction between CDT1 and the histone H4 acetylase HBO1 to replication origins. Loss of this interaction abrogates the acetylation required for replication initiation. Promotes stressed cell apoptosis by phosphorylating key regulatory factors including p53/TP53 and Yes-associates protein YAP1. In T-cells, MAPK8 and MAPK9 are required for polarized differentiation of T-helper cells into Th1 cells. Contributes to the survival of erythroid cells by phosphorylating the antagonist of cell death BAD upon EPO stimulation. Mediates starvation-induced BCL2 phosphorylation, BCL2 dissociation from BECN1, and thus activation of autophagy. Phosphorylates STMN2 and hence regulates microtubule dynamics, controlling neurite elongation in cortical neurons. In the developing brain, through its cytoplasmic activity on STMN2, negatively regulates the rate of exit from multipolar stage and of radial migration from the ventricular zone. Phosphorylates several other substrates including heat shock factor protein 4 (HSF4), the deacetylase SIRT1, ELK1, or the E3 ligase ITCH. JNK1 isoforms display different binding patterns: beta-1 preferentially binds to c-Jun, whereas alpha-1, alpha-2, and beta-2 have a similar low level of binding to both c-Jun or ATF2. However, there is no correlation between binding and phosphorylation, which is achieved at about the same efficiency by all isoforms.

Sequence similarities Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase

subfamily.

Contains 1 protein kinase domain.

Domain The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the

MAP kinases.

Post-translational

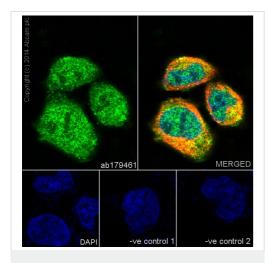
modifications

Dually phosphorylated on Thr-183 and Tyr-185 by MAP2K7 and MAP2K4, which activates the

enzyme. Phosphorylated by TAOK2.

Cellular localization Cytoplasm. Nucleus.

Images



Immunocytochemistry/ Immunofluorescence - Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling JNK1+JNK2+JNK3 with ab179461 at 1/250 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/400 dilution (green). Confocal image showing both cytoplasmic and nuclear staining on HeLa cells. The nuclear counterstain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/500 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:-

-ve control 1: - ab179461 at 1/250 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: - <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/500 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/400 dilution.

1 2 3
250 KDa —
150 KDa —
100 KDa —
75 KDa —
50 KDa —
50 KDa —
4 JNK p54
4 JNK p46
4 JNK p46

Western blot - Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461)

All lanes : Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461) at 1/20000 dilution

Lane 1 : K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysates

Lane 2: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates

Lane 3 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysates

Lysates/proteins at 20 µg per lane.

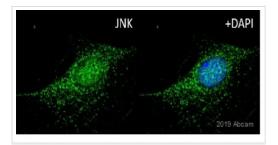
Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 48 kDa **Observed band size:** 46,54 kDa

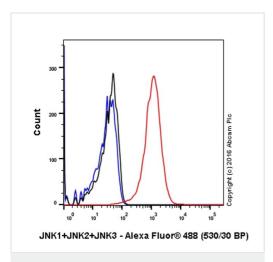
Blocking/Dilution buffer: 5% NFDM/TBST.

JNKs originate from three genes that yield ten isoforms through alternative mRNA splicing, including JNK1 α 1, JNK1 β 1, JNK2 α 1, JNK2 β 1 and JNK3 α 1, which represent the p46 isoforms, and JNK1 α 2, JNK1 β 2, JNK2 α 2, JNK2 β 2 and JNK3 β 2, which represent the p54 isoforms.



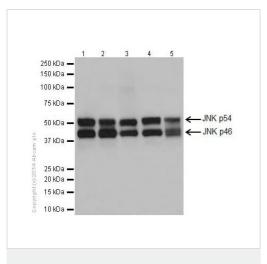
Immunocytochemistry/ Immunofluorescence - Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461)

Formaldehyde-fixed, NP40 permeabilized Mouse Vascular smooth muscle cells stained for JNK1+JNK2+JNK3 (Green) using ab179461 at 1/200 dilution followed by a Donkey anti-rabbit Alex Fluor® 488 antibody at 1/500 dilution. The nuclear counterstain was DAPI (Blue).



Flow Cytometry (Intracellular) - Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling JNK1+JNK2+JNK3 with purified ab179461 at 1/180 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Western blot - Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461)

All lanes : Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461) at 1/5000 dilution

Lane 1: Neuro-2a (Mouse neuroblastoma cells) whole cell lysates

Lane 2 : UMNSAH/DF-1 (Transformed chicken embyronic

fibroblast cells) whole cell lysates

Lane 3: MDCK (Canine kidney cell line) whole cell lysates

Lane 4: MDBK (Bovine kidney cell line) whole cell lysates

Lane 5 : COS-1 (African green monkey kidney fibroblast-like cell

line) whole cell lysates

Lysates/proteins at 10 µg per lane.

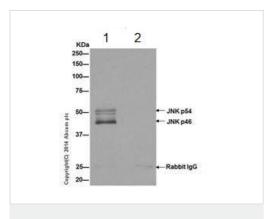
Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 48 kDa Observed band size: 46,54 kDa

Blocking/dilution buffer: 5% NFDM/TBST.

JNKs originate from three genes that yield ten isoforms through alternative mRNA splicing, including JNK1 α 1, JNK1 β 1, JNK2 α 1, JNK2 β 1 and JNK3 α 1, which represent the p46 isoforms, and JNK1 α 2, JNK1 β 2, JNK2 α 2, JNK2 β 2 and JNK3 β 2, which represent the p54 isoforms.



Immunoprecipitation - Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461)



Western blot - Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461)

JNK1+JNK2+JNK3 were immunoprecipitated from 1mg of Jurkat (Human T cell leukemia cells from peripheral blood) whole cell extract with ab179461 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab179461 at 1/1000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution. Lane 1: Jurkat whole cell extract. Lane 2: PBS instead of Jurkat whole cell extract.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

JNKs originate from three genes that yield ten isoforms through alternative mRNA splicing, including JNK1 α 1, JNK1 β 1, JNK2 α 1, JNK2 β 1 and JNK3 α 1, which represent the p46 isoforms, and JNK1 α 2, JNK1 β 2, JNK2 α 2, JNK2 β 2 and JNK3 β 2, which represent the p54 isoforms.

All lanes: Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461) at 1/20000 dilution

Lane 1 : Human JNK3 full length recombinant protein containing a proprietary tag.

Lane 2: Human JNK2 full length recombinant protein containing a proprietary tag.

Lane 3 : Human JNK1 full length recombinant protein containing a His tag.

Lysates/proteins at 0.01 µg per lane.

Secondary

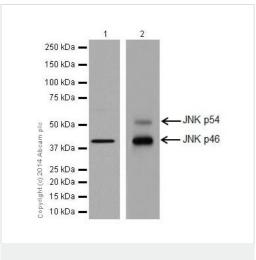
All lanes : Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 48 kDa

Additional bands at: 48 kDa (possible tagged protein), 71 kDa (possible tagged protein), 71 kDa (possible tagged protein)

Human JNK1, JNK2 and JNK3 full length recombinant proteins are from commercial sources. JNK1 and JNK2 have a proprietary tag, JNK3 has a His tag.

Blocking/dilution buffer: 5% NFDM/TBST.



Western blot - Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461)

All lanes: Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461) at 1/1000 dilution

Lane 1 : Zebrafish lysate

Lane 2 : X. tropicalis lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 48 kDa **Observed band size:** 46,54 kDa

Blocking/dilution buffer: 5% NFDM/TBST.

Zebrafish has only one JNK isoform, JNK1 with a MW of 44kDa. So there is only one band in Zebrafish.

1 2 3 4 5 6

250 kDa —

150 kDa —

100 kDa —

75 kDa —

50 kDa —

37 kDa —

25 kDa —

20 kDa —

15 kDa —

15 kDa —

10 kDa —

10 kDa —

10 kDa —

Western blot - Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461)

All lanes : Anti-JNK1 + JNK2 + JNK3 antibody [EPR16797-211] (ab179461) at 1/5000 dilution

Lane 1: Mouse brain lysate

Lane 2: Rat brain lysate

Lane 3: Rat heart lysate

Lane 4: RAW 264.7 (Mouse macrophage cells transformed with

Abelson murine leukemia virus) lysate

Lane 5: PC-12 (Rat adrenal gland pheochromocytoma) lysate

Lane 6: NIH/3T3 (Mouse embyro fibroblast cells) lysate

Lysates/proteins at 10 µg per lane.

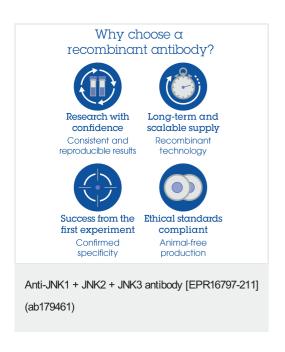
Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 48 kDa **Observed band size:** 46,54 kDa

Blocking/dilution buffer: 5% NFDM/TBST.

JNKs originate from three genes that yield ten isoforms through alternative mRNA splicing, including JNK1 α 1, JNK1 β 1, JNK2 α 1, JNK2 β 1 and JNK3 α 1, which represent the p46 isoforms, and JNK1 α 2, JNK1 β 2, JNK2 α 2, JNK2 β 2 and JNK3 β 2, which represent the p54 isoforms.



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