abcam

Product datasheet

Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] ab208035



Overview

Product name Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95]

Description Rabbit monoclonal [EPR18841-95] to JNK1 + JNK2 + JNK3

Host species Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human

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; Human fetal liver, fetal heart

and fetal kidney lysates; Jurkat, HeLa, K562, MCF7, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates; Mouse brain, heart, kidney and spleen lysates; Rat brain, kidney and spleen lysates. ICC/IF: HeLa and NIH/3T3 cell lines. Flow Cyt (intra): Jurkat and HeLa cell lines. IP: HeLa whole

cell lysate.

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 pH: 7.2

Preservative: 0.01% Sodium azide

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

Purity Protein A purified

1

Clonality Monoclonal
Clone number EPR18841-95

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise quarantee covers the use of ab208035 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	****(1)	1/2000. Detects a band of approximately 46-54 kDa (predicted molecular weight: 48, 53 kDa).
ICC/IF		1/1000.
IP		1/40.
Flow Cyt (Intra)		1/100.

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

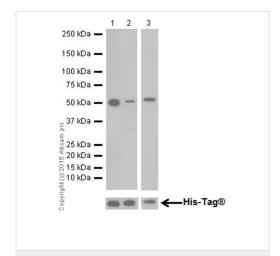
modifications

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

enzyme. Phosphorylated by TAOK2.

Cellular localization Cytoplasm. Nucleus.

Images



Western blot - Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035) **All lanes :** Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035) at 1/2000 dilution

Lane 1: Human JNK1 full length recombinant protein

Lane 2: Human JNK2 full length recombinant protein

Lane 3: Human JNK3 full length recombinant protein

Lysates/proteins at 0.01 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

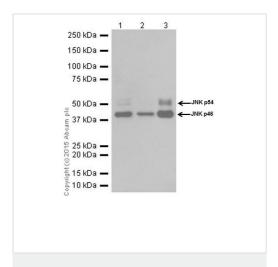
Predicted band size: 48, 53 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 & 2: 5 seconds; Lane 3: 15 seconds.

Human JNK1 full length recombinant protein contains aa1-427 with a His-Tag®. Human JNK2 full length recombinant protein contains aa1-424 with a His-Tag®. Human JNK3 full length recombinant protein contains aa1-464 with a His-Tag®.

All three recombinant human full length proteins were made inhouse.



Western blot - Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035) **All lanes :** Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035) at 1/2000 dilution

Lane 1: Human fetal liver lysate

Lane 2: Human fetal heart lysate

Lane 3: Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

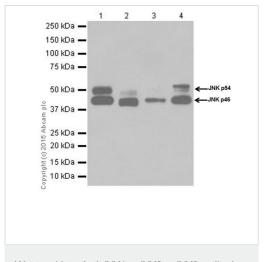
Secondary

All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 48, 53 kDa **Observed band size:** 46-54 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035)

All lanes : Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035) at 1/2000 dilution

Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 3 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

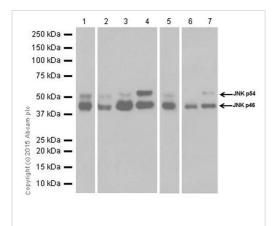
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 48, 53 kDa **Observed band size:** 46-54 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035)

All lanes : Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035) at 1/2000 dilution

(ab208035) at 1/2000 dilution

Lane 2 : Mouse heart lysate
Lane 3 : Mouse kidney lysate
Lane 4 : Mouse spleen lysate
Lane 5 : Rat brain lysate
Lane 6 : Rat kidney lysate

Lane 1: Mouse brain lysate

Lysates/proteins at 10 µg per lane.

Secondary

Lane 7: Rat spleen lysate

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 48, 53 kDa Observed band size: 46-54 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 4 seconds; Lane 2,3 & 4: 30 seconds; Lane

5: 15 seconds; Lane 6 & 7: 3 minutes.

All lanes: Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035) at 1/2000 dilution

Lane 1: C6 (Rat glial tumor cell line) whole cell lysate

Lane 2: RAW 264.7 (Mouse macrophage cell line transformed

with Abelson murine leukemia virus) whole cell lysate

Lane 3: PC-12 (Rat adrenal gland pheochromocytoma cell line)

whole cell lysate

Lane 4: NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell

lysate

Lysates/proteins at 10 µg per lane.

1 2 3 4

50 kDa —

Western blot - Anti-JNK1 + JNK2 + JNK3 antibody

250 kDa -

150 kDa 🕳 100 kDa -75 kDa 🕳

្ទឹ 37 kDa 🕳

25 kDa — 20 kDa —

ਦੂ 15 kDa 🕳

10 kDa —

[EPR18841-95] (ab208035)

Secondary

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

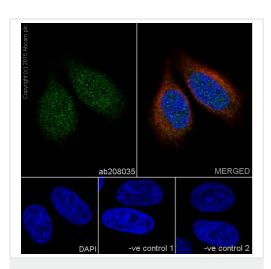
1/100000 dilution

Predicted band size: 48, 53 kDa

Observed band size: 46-54 kDa

Exposure time: 8 seconds

Blocking and Diluting buffer and concentration: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cell line labeling JNK1+JNK2+JNK3 with ab208035 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

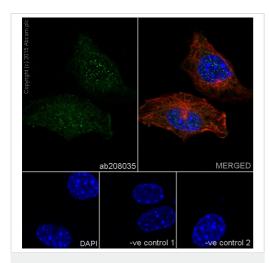
Confocal image showing cytoplasm and weak nuclear staining on HeLa cell line.

The nuclear counterstain is DAPI (blue).

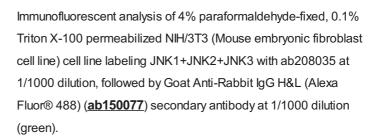
Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) at 1/1000 dilution and Goat Anti-Mouse lgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) at 1/1000 dilution (red).

The negative controls are as follows:-

- -ve control 1: ab208035 at 1/1000 dilution followed by **ab150120** at 1/1000 dilution.
- -ve control 2: <u>ab7291</u> at 1/1000 dilution followed by <u>ab150077</u> at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035)



Confocal image showing cytoplasm and nuclear staining on NIH/3T3 cell line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) at 1/1000 dilution and Goat Anti-Mouse lgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) at 1/1000 dilution (red).

The negative controls are as follows:-

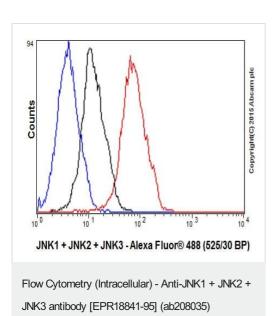
- -ve control 1: ab208035 at 1/1000 dilution followed by <u>ab150120</u> at 1/1000 dilution.
- -ve control 2: $\underline{ab7291}$ at 1/1000 dilution followed by $\underline{ab150077}$ at 1/1000 dilution.

JNK1 + JNK2 + JNK3 - Alexa Fluor® 488 (525/30 BP)

Flow Cytometry (Intracellular) - Anti-JNK1 + JNK2 + JNK3 antibody [EPR18841-95] (ab208035)

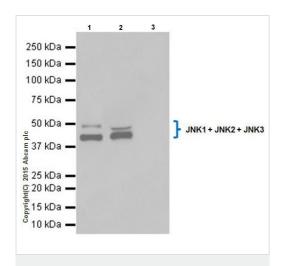
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed Jurkat (Human T cell leukemia cell line from peripheral blood) cell linelabeling JNK1+JNK2+JNK3 with ab208035 at 1/100 dilution (red) compared with a Rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue).

Goat anti-Rabbit IgG (Alexa Fluor[®] 488) at 1/500 dilution was used as the secondary antibody.



Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cell linelabeling JNK1+JNK2+JNK3with ab208035 at 1/100 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue).

Goat anti-Rabbit lgG (Alexa Fluor[®] 488) at 1/500 dilution was used as the secondary antibody.



JNK1+JNK2+JNK3 was immunoprecipitated from 1mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab208035 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab208035 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

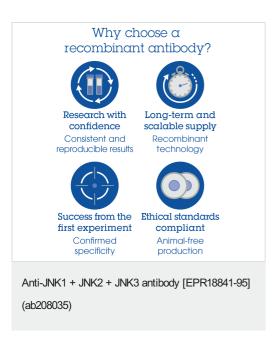
Lane 1: HeLa whole cell lysate, 10ug (Input).

Lane 2: ab208035 IP in HeLa whole cell lysate.

Lane 3: Rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) instead of ab208035 in HeLa whole cell lysate.

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

Exposure time: 10 seconds.



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 https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

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