abcam

Product datasheet

Anti-JNK2 antibody [EP1595Y] ab76125





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Overview

Product name Anti-JNK2 antibody [EP1595Y]

Description Rabbit monoclonal [EP1595Y] to JNK2

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IP, IHC-P, ELISA

Species reactivity Reacts with: Human, Recombinant fragment

Predicted to work with: Mouse, Rat

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

Positive control WB: HEK293T, MCF7, HAP1 and HeLa cell lysates. IHC-P: Human breast carcinoma tissue. IP:

HeLa cell lysate. Flow Cyt (intra): HeLa cells.

General notes 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**.

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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EP1595Y

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab76125 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)		1/40. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	*** <u>*</u>	1/1000 - 1/10000. Predicted molecular weight: 48 kDa.
IP		Use a concentration of 5 µg/ml.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ELISA		Use at an assay dependent concentration.

Target

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Responds to activation by environmental stress and pro-inflammatory cytokines by phosphorylating a number of transcription factors, primarily components of AP-1 such as c-Jun

and ATF2 and thus regulates AP-1 transcriptional activity. In T-cells, JNK1 and JNK2 are required

for polarized differentiation of T-helper cells into Th1 cells.

JNK2 isoforms display different binding patterns: alpha-1 and alpha-2 preferentially bind to c-Jun, whereas beta-1 and beta-2 bind to ATF2. However, there is no correlation between binding and phosphorylation, which is achieved at about the same efficiency by all isoforms. JUNB is not a

substrate for JNK2 alpha-2, and JUND binds only weakly to it.

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

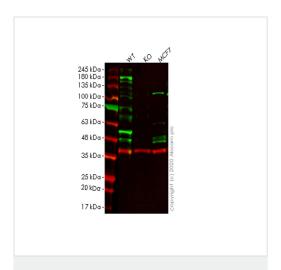
MAP kinases.

Post-translational modifications

Dually phosphorylated on Thr-183 and Tyr-185, which activates the enzyme. Autophosphorylated

in vitro.

Images



Western blot - Anti-JNK2 antibody [EP1595Y] (ab76125)

All lanes : Anti-JNK2 antibody [EP1595Y] (ab76125) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: MAPK9 knockout HEK293T cell lysate

Lane 3: MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 48 kDa **Observed band size:** 48 kDa

Lanes 1-3: Merged signal (red and green). Green - ab76125 observed at 48 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

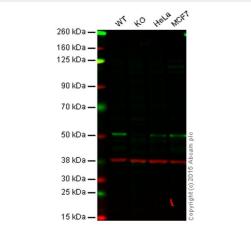
ab76125 Anti-JNK2 antibody [EP1595Y] was shown to specifically react with JNK2 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266355 (knockout cell lysate ab257527) was used. Wild-type and JNK2 knockout samples were subjected to SDS-PAGE. ab76125 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



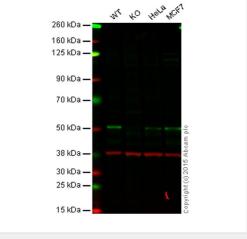
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-JNK2 antibody [EP1595Y] (ab76125)

ab76125 at 1/100 dilution staining JNK2 in human breast carcinoma by Immunohistochemistry, Paraffin-embedded tissue.

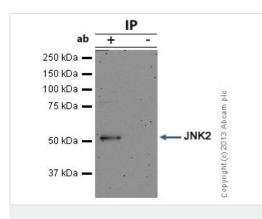
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-JNK2 antibody [EP1595Y]



(ab76125)



Immunoprecipitation - Anti-JNK2 antibody [EP1595Y] (ab76125)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: JNK2 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: MCF7 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab76125 observed at 54 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab76125 was shown to specifically react with JNK2 when JNK2 knockout samples were used. Wild-type and JNK2 knockout samples were subjected to SDS-PAGE. ab76125 and ab8245 (loading control to GAPDH) were diluted 1/2500 and 1/2000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.

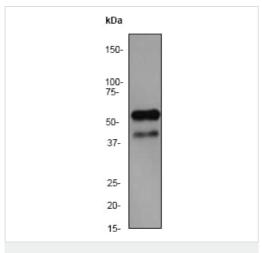
JNK2 was immunoprecipitated using 0.5mg Hela whole cell extract, 5μg of Rabbit monoclonal to JNK2 and 50μl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab76125.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).

Band: 48kDa; JNK2



Western blot - Anti-JNK2 antibody [EP1595Y] (ab76125)

Anti-JNK2 antibody [EP1595Y] (ab76125) at 1/50000 dilution + HeLa cell lysate at 10 μg

Secondary

goat anti-rabbit-HRP at 1/1000 dilution

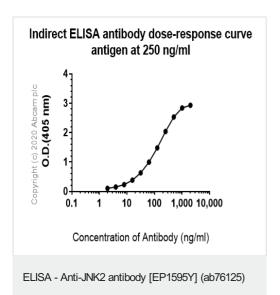
Developed using the ECL technique.

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

Additional bands at: 46 kDa (possible isoform)

Flow Cytometry (Intracellular) - Anti-JNK2 antibody [EP1595Y] (ab76125)

Overlay histogram showing HeLa cells stained with ab76125 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab76125, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x106 cells) used under the same conditions. Acquisition of >5,000 events was performed.



ELISA analysis of Human JNK2 recombinant protein at 250 ng/mL with ab76125. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution was used as the secondary antibody.



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