abcam

Product datasheet

Anti-JNK2 antibody [EP1595Y] - BSA and Azide free ab227986

KO VALIDATED Recombinant RabMAb

10 References 7 Images

Overview

Product name	Anti-JNK2 antibody [EP1595Y] - BSA and Azide free
Description	Rabbit monoclonal [EP1595Y] to JNK2 - BSA and Azide free
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,Â MCF7, HAP1 and HeLa cell lysates. IP: HeLa cell lysate. Flow Cyt (intra): HeLa cells. IHC-P: Human breast carcinoma tissue.
General notes	ab227986 is the carrier-free version of **ab76125**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20

	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1595Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227986 in the following tested applications.

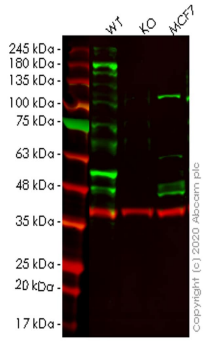
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Predicted molecular weight: 48 kDa.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ELISA		Use at an assay dependent concentration.

Target

Function	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] - BSA and Azide free (ab227986)

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.

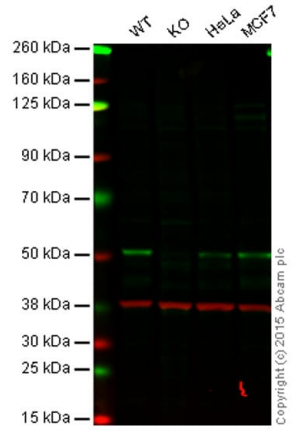
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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

This data was developed using the same antibody clone in a different buffer formulation (**ab76125**).

Lanes 1-3: Merged signal (red and green). Green - **ab76125** observed at 48 kDa. Red - loading control **ab8245** 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.

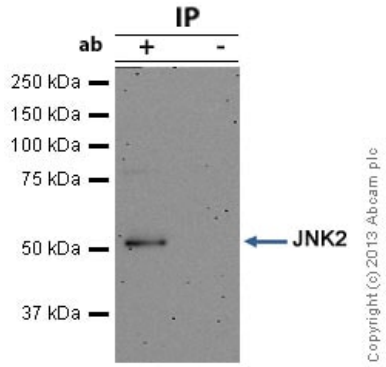


Western blot - Anti-JNK2 antibody [EP1595Y] - BSA and Azide free (ab227986)

This WB data was generated using the same anti-JNK2 antibody clone, EP1595Y, in a different buffer formulation (cat# **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 IgG 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.



Immunoprecipitation - Anti-JNK2 antibody [EP1595Y] - BSA and Azide free (ab227986)

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.

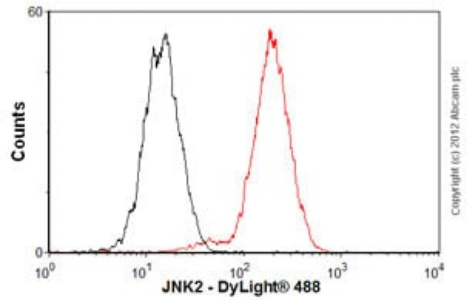
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with **ab76125**.

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

Band: 48kDa; JNK2

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

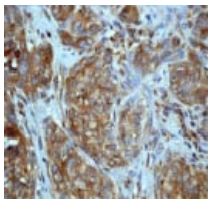
sodium azide (**ab76125**).



Flow Cytometry (Intracellular) - Anti-JNK2 antibody [EP1595Y] - BSA and Azide free (ab227986)

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 IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab76125**).

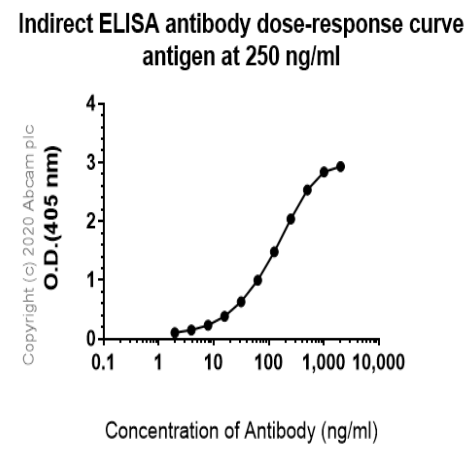


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-JNK2 antibody [EP1595Y] - BSA and Azide free (ab227986)

This IHC data was generated using the same anti-JNK2 antibody clone, EP1595Y, in a different buffer formulation (cat# **ab76125**).

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

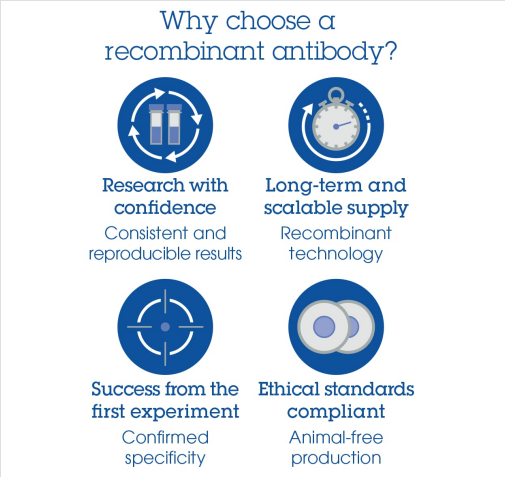
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



ELISA - Anti-JNK2 antibody [EP1595Y] - BSA and Azide free (ab227986)

This data was developed using **ab76125**, the same antibody clone in a different buffer formulation.

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.



Anti-JNK2 antibody [EP1595Y] - BSA and Azide free

(ab227986)

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