abcam

Product datasheet

Anti-TAK1 antibody [EPR5984] - BSA and Azide free ab222394

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-TAK1 antibody [EPR5984] - BSA and Azide free
Description	Rabbit monoclonal [EPR5984] to TAK1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293T, K562, HeLa and A431 cell lysates. IHC-P: Human brain tissue. ICC/IF: Wild-type HAP1 cells.
General notes	ab222394 is the carrier-free version of **ab109526**. 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. 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. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5984
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab222394 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, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 75 kDa (predicted molecular weight: 67 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol. (Heat to 98°C, allow to cool for 10-20 minutes)
ICC/IF		Use at an assay dependent concentration.

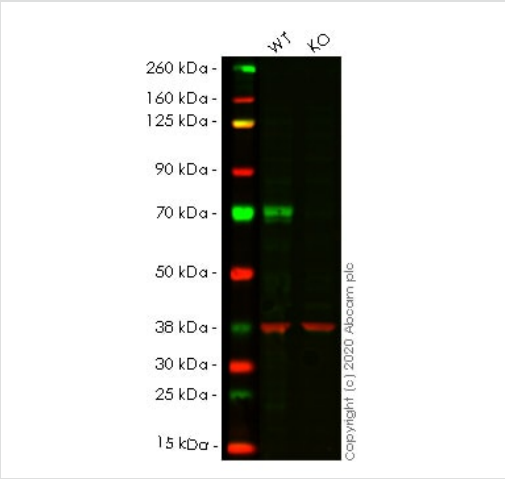
Application notes Is unsuitable for IP.

Target

Function	Component of a protein kinase signal transduction cascade. Mediator of TRAF6 and TGF-beta signal transduction. Activates IKBKB and MAPK8 in response to TRAF6 signaling. Stimulates NF-kappa-B activation and the p38 MAPK pathway. In osmotic stress signaling, plays a major role in the activation of MAPK8/JNK, but not that of NF-kappa-B.
Sequence similarities	Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. MAP kinase kinase kinase subfamily. Contains 1 protein kinase domain.
Post-translational modifications	Association with TAB1/MAP3K7IP1 promotes autophosphorylation and subsequent activation. Association with TAB2/MAP3K7IP2, itself associated with free unanchored Lys-63 polyubiquitin chain, promotes autophosphorylation and subsequent activation of MAP3K7. Dephosphorylation at Thr-187 by PP2A and PPP6C leads to inactivation. Ubiquitinated, leading to proteasomal degradation (By similarity). Requires 'Lys-63'-linked

polyubiquitination for autophosphorylation and subsequent activation. 'Lys-63'-linked ubiquitination does not lead to proteasomal degradation. Deubiquitinated by CYLD, a protease that selectively cleaves 'Lys-63'-linked ubiquitin chains. Deubiquitinated by Y.enterocolitica YopP.

Images



Western blot - Anti-TAK1 antibody [EPR5984] - BSA and Azide free (ab222394)

All lanes : Anti-TAK1 antibody [EPR5984] (**ab109526**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : MAP3K7 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

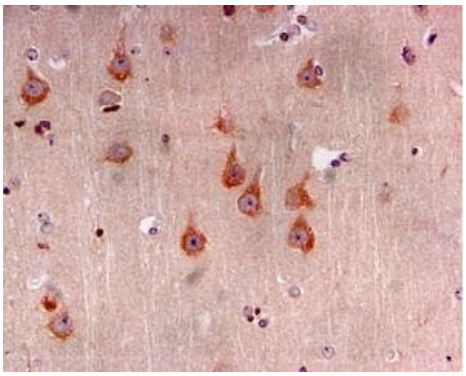
Performed under reducing conditions.

Predicted band size: 67 kDa
Observed band size: 72 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab109526**).

Lanes 1- 2: Merged signal (red and green). Green - **ab109526** observed at 72 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab109526 was shown to react with TAK1 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266555** (knockout cell lysate **ab256984**) was used. Wild-type HEK-293T and MAP3K7 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab109526** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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.

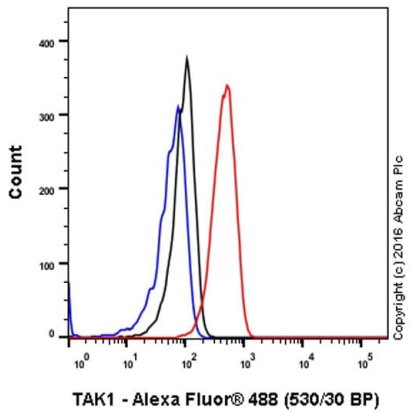


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TAK1 antibody [EPR5984] - BSA and Azide free (ab222394)

ab109526, at a 1/50 dilution, staining TAK1 in Formalin/PFA-fixed paraffin-embedded Human brain tissue, by Immunohistochemistry.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109526**).

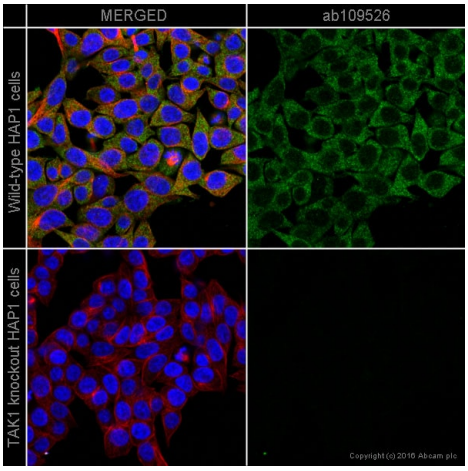
Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-TAK1 antibody [EPR5984] - BSA and Azide free (ab222394)

Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labeling TAK1 with unpurified **ab109526** at 1/20 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109526**).

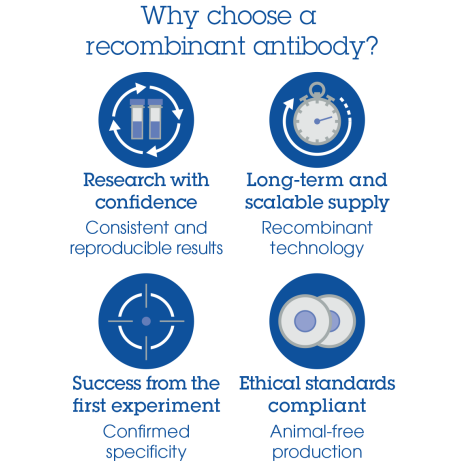


Immunocytochemistry/ Immunofluorescence - Anti-TAK1 antibody [EPR5984] - BSA and Azide free (ab222394)

ab109526 staining TAK1 in wild-type HAP1 cells (top panel) and TAK1 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), 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 **ab109526** at 1/1000 dilution and **ab195889** at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109526**).



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