abcam

Product datasheet

# Anti-TAK1 antibody [EPR5984] ab109526

KO VALIDATED Recombinant RabMAb

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## Overview

| | |
|---|---|
| **Product name** | Anti-TAK1 antibody [EPR5984] |
| **Description** | Rabbit monoclonal [EPR5984] to TAK1 |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF<br>**Unsuitable for:** IP |
| **Species reactivity** | **Reacts with:** Human<br>**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** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>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. Stable for 12 months at -20°C. |
| **Storage buffer** | pH: 7.20<br>Preservative: 0.05% Sodium azide<br>Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |

| | |
|---|---|
| **Clone number** | EPR5984 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab109526 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.<br>**ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |
| **WB** | ★★★★☆ **(2)** | 1/1000 - 1/10000. Detects a band of approximately 75 kDa (predicted molecular weight: 67 kDa). |
| **IHC-P** | | 1/50 - 1/100. 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** | | 1/1000. |

**Application notes** Is unsuitable for IP.

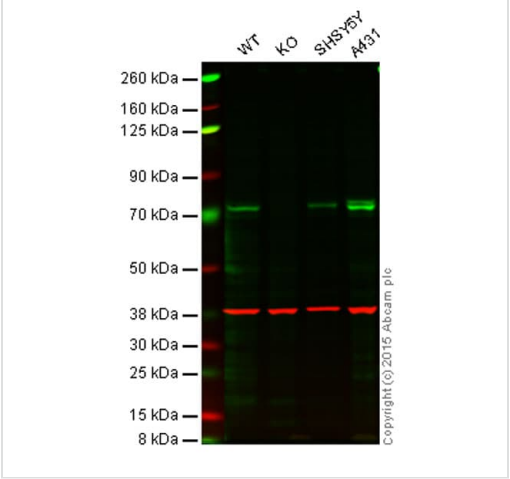
## Target

**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.

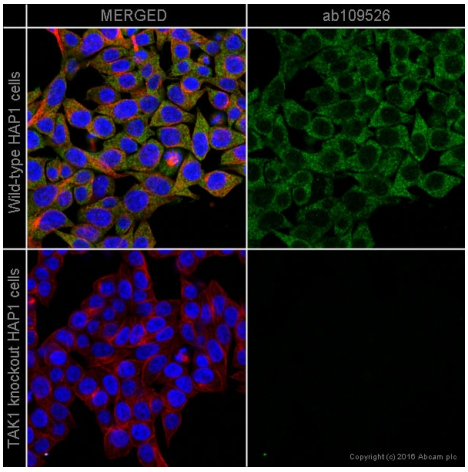
**Post-translational modifications** Association with TAB1/MAP3K7IP1 promotes autophosphorylation and subsequent activation. Association with TAB2/MAP3K7IP2, itself associated with free unanchored Lys-63 polyubiquitin chain, promotes autophosphorylation and subsequent activation of MAP3K7. Dephosphorylation 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] (ab109526)

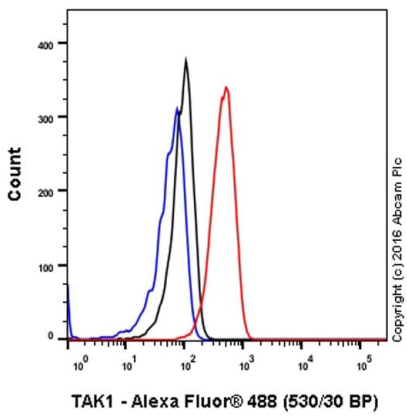
**Lane 1**: Wild-type HAP1 cell lysate (20 µg)
**Lane 2**: TAK1 knockout HAP1 cell lysate (20 µg)
**Lane 3**: SHSY5Y cell lysate (20 µg)
**Lane 4**: A431 cell lysate (20 µg)
**Lanes 1 - 4**: Merged signal (red and green). Green - ab109526 observed at 72 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab109526 was shown to specifically react with TAK1 when TAK1 knockout samples were used. Wild-type and TAK1 knockout samples were subjected to SDS-PAGE. ab109526 and **ab8245** (loading control to GAPDH) were diluted 1/1000 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 1/10 000 dilution for 1 h at room temperature before imaging.




Immunocytochemistry/ Immunofluorescence - Anti-TAK1 antibody [EPR5984] (ab109526)

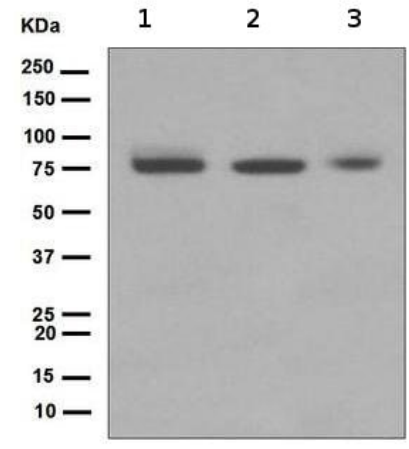
ab109526 staining TAK1 in wild-type HAP1 cells (top panel) and TAK1 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells 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).




Flow Cytometry (Intracellular) - Anti-TAK1 antibody [EPR5984] (ab109526)

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.




Western blot - Anti-TAK1 antibody [EPR5984] (ab109526)

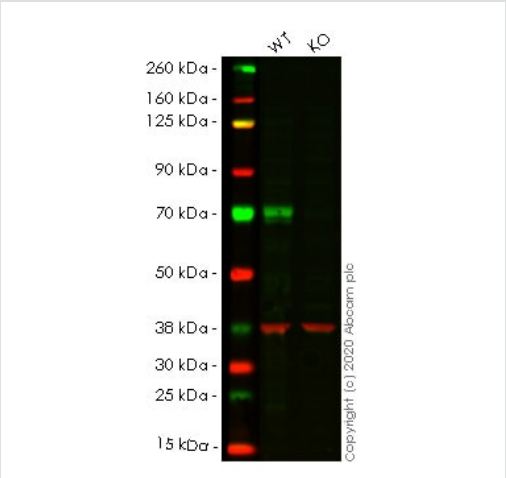
**All lanes :** Anti-TAK1 antibody [EPR5984] (ab109526) at 1/1000 dilution

**Lane 1 :** K562 cell lysate
**Lane 2 :** HeLa cell lysate
**Lane 3 :** A431 cell lysate

Lysates/proteins at 10 µg per lane.

**Secondary**
**All lanes :** HRP labelled Goat anti-Rabbit IgG at 1/2000 dilution

**Predicted band size:** 67 kDa
**Observed band size:** 75 kDa




Western blot - Anti-TAK1 antibody [EPR5984] (ab109526)

**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

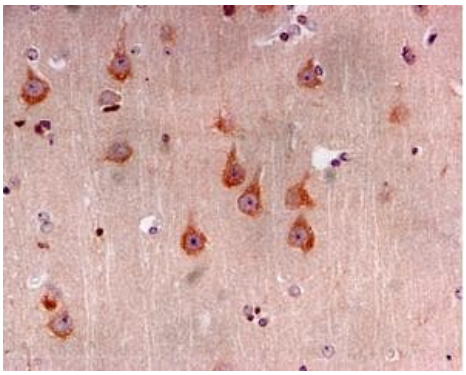
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

**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] (ab109526)

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

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


Anti-TAK1 antibody [EPR5984] (ab109526)



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