abcam

Product datasheet

Anti-NAK/TBK1 antibody [EP611Y] ab40676

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-NAK/TBK1 antibody [EP611Y]
Description	Rabbit monoclonal [EP611Y] to NAK/TBK1
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human NAK/TBK1 aa 1-100 (N terminal). The exact sequence is proprietary.
Positive control	WB: HeLa membrane extract lysate (**ab29547**), HepG2, SH-SY5Y, C6, HAP1 and NIH/3T3 cell lysate IHC-P: Human hepatocellular carcinoma ICC/IF: MCF7 cells
General notes	Anti-NAK/TBK1 antibody [EP611Y] (ab40676) may not be suitable for IHC with mouse or rat samples. 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: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EP611Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40676 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
ICC/IF		1/100. **For unpurified use at 1/250 - 1/500.**
WB	★★★★★ **(2)**	1/5000. Detects a band of approximately 84 kDa (predicted molecular weight: 84 kDa). **For unpurified use at 1/1000.**
IHC-P	★★★★☆ **(1)**	1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. **For unpurified use at 1/50.**

Target

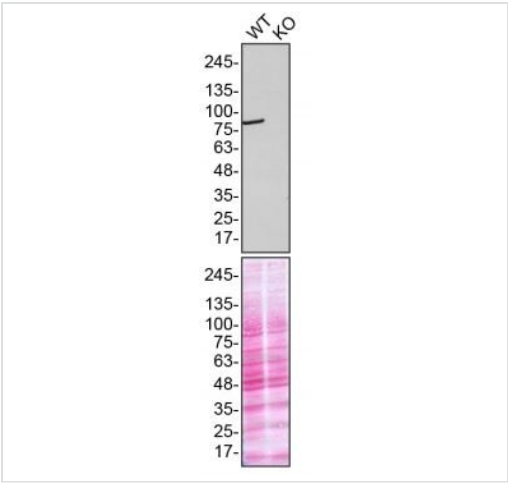
Function Serine/threonine protein involved in the signaling cascade converging to the activation of the transcription factor NF-kappa-B. May function as an IKK kinase, playing an essential role in the transcription of a subset of TNF-alpha-induced genes. Also mediates production of RANTES/CCL5 and interferon-beta/IFNB1. Has a pivotal role in the innate immune response. Phosphorylates Borna disease virus (BDV) P protein. Phosphorylates and activates IRF3 and IRF7 and allows their nuclear localization. This leads to production of alpha/beta interferons and the development of a cellular antiviral state. It also seems to be a central factor in the induction of the antiviral interferon response. Inhibition of its interaction with IRF3, due to HCV NS3 binding or BDV P protein seems to be one mechanism of inhibition of the innate immune responses of hepatitis C virus (HCV) infection or Borna disease virus infection respectively.

Tissue specificity Ubiquitous with higher expression in testis.

Sequence similarities Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. I-kappa-B kinase subfamily.
Contains 1 protein kinase domain.

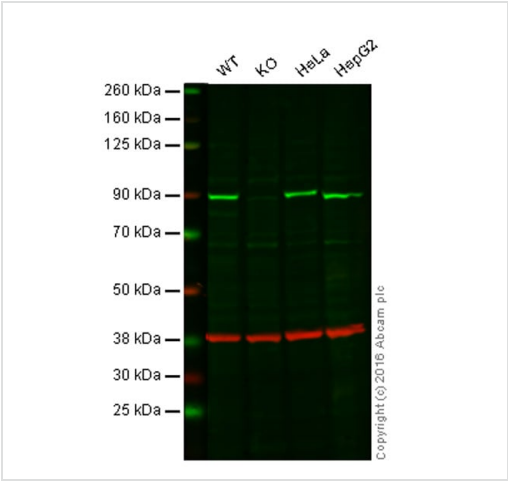
Cellular localization Cytoplasm.

Images



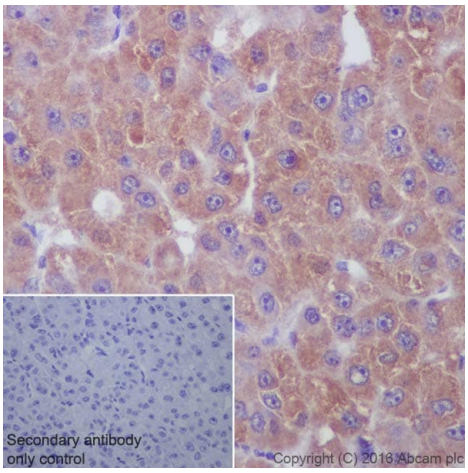
Western blot - Anti-NAK/TBK1 antibody [EP611Y] (ab40676)

ab40676 was shown to react with TBK1 in wild-type U2OSn cells in Western blot with loss of signal observed in a TBK1 knockout cell line. Wild-type U2OSn and TBK1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab40676 overnight at 4 °C at a 1/10000 dilution. Blots were incubated with goat anti-rabbit HRP secondary antibodies at 1/5000 before imaging. These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



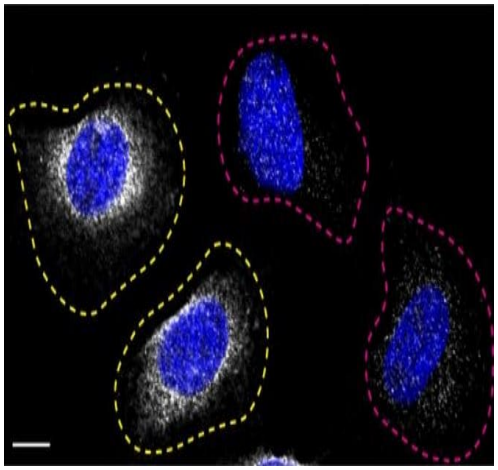
Western blot - Anti-NAK/TBK1 antibody [EP611Y] (ab40676)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: NAK knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab40676 observed at 90 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab40676 was shown to specifically react with NAK when NAK knockout samples were used. Wild-type and NAK knockout samples were subjected to SDS-PAGE. ab40676 and **ab8245** (loading control to GAPDH) were diluted at 1/1000 and 1/10,000 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NAK/TBK1 antibody [EP611Y] (ab40676)

Immunohistochemical analysis of paraffin-embedded human hepatocellular carcinoma tissue sections labeling NAK/TBK1 with purified ab40676 at a dilution of 1/100 (11.5 µg/ml). **ab97051** Goat Anti-Rabbit IgG H&L (HRP) at 1/500 was used as the secondary anitbody. Sections were counterstained with hematoxylin. Antigen retrieval was heat mediated using EDTA Buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.

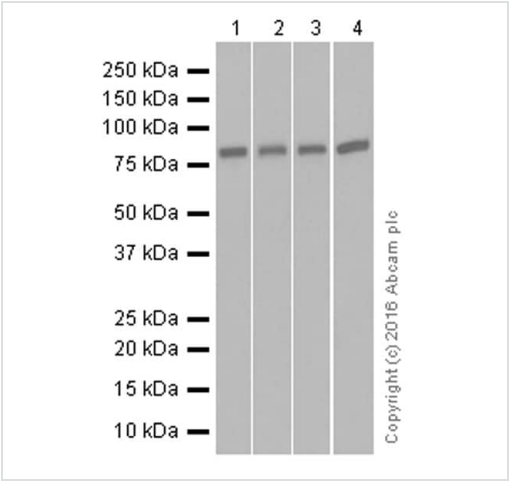


Immunocytochemistry/ Immunofluorescence - Anti-NAK/TBK1 antibody [EP611Y] (ab40676)

ab40676 was shown to react with TBK1 in wild-type U2OSn cells in Immunocytochemistry with loss of signal observed in a TBK1 knockout cell line. Wild-type and Knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 5%BSA+5%goat serum (30min). The cells were then incubated with ab40676 at 1/1500 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880).

These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by

enabling the life science community to better evaluate commercially available antibodies.



Western blot - Anti-NAK/TBK1 antibody [EP611Y] (ab40676)

All lanes : Anti-NAK/TBK1 antibody [EP611Y] (ab40676) at 1/5000 dilution (purified)

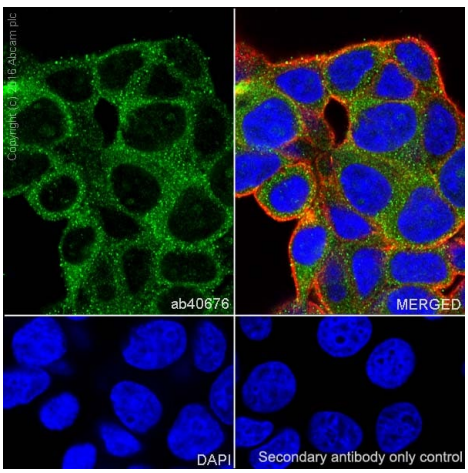
Lane 1 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 2 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate
Lane 3 : C6 (Rat glial tumor cell line) whole cell lysate
Lane 4 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 84 kDa
Observed band size: 84 kDa

Blocking/Diluting buffer 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-NAK/TBK1 antibody [EP611Y] (ab40676)

Immunocytochemistry/Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma) cells labeling NAK/TBK1 with purified ab40676 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, Alexa Fluo®488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were counter-stained with **ab195889** Anti-Alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor®594) at 1/200. DAPI (blue) was used as a nuclear counterstain. Secondary Only Control: PBS was used instead of the primary antibody as the negative control.

Anti-NAK/TBK1 antibody [EP611Y] (ab40676)

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