abcam

Product datasheet

Anti-NAK/TBK1 antibody [EPR2867(2)-19] ab109735





★★★★★ 1 Abreviews 17 References 8 Images

Overview

Product name Anti-NAK/TBK1 antibody [EPR2867(2)-19]

Description Rabbit monoclonal [EPR2867(2)-19] to NAK/TBK1

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, IP

Unsuitable for: Flow Cyt

Species reactivity Reacts with: Human

Predicted to work with: Rat 4

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

Positive control HeLa, U937, HepG2, and 293T cell lysates, Human testis tissue, 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 -20°C. Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR2867(2)-19

Isotype ΙgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab109735 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
WB	****(1)	1/1000 - 1/10000. Detects a band of approximately 84 kDa (predicted molecular weight: 84 kDa). For unpurified use at 1/1000 - 1/2000.
IHC-P		1/800. 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 1/400.
IP		1/30.

Application notes

Is unsuitable for Flow Cyt.

Target

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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

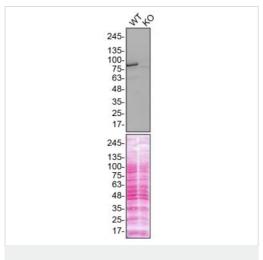
subfamily.

Contains 1 protein kinase domain.

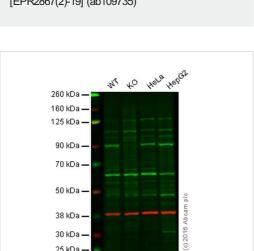
Cellular localization

Cytoplasm.

Images



Western blot - Anti-NAK/TBK1 antibody [EPR2867(2)-19] (ab109735)



Western blot - Anti-NAK/TBK1 antibody [EPR2867(2)-19] (ab109735)

ab109735 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 ab109735 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.

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

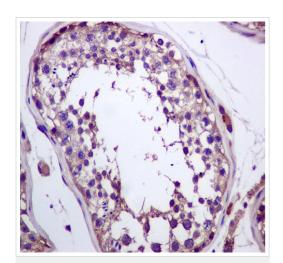
Lane 2: NAK/TBK1 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: HepG2 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab109735 observed at 90 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

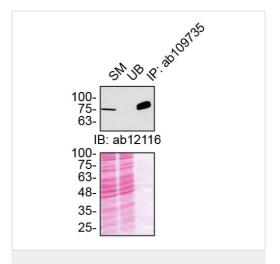
ab109735 was shown to recognize NAK/TBK1 when NAK/TBK1 knockout samples were used, along with additional cross-reactive bands. Wild-type and NAK/TBK1 knockout samples were subjected to SDS-PAGE. ab109735 and ab8245 (loading control to GAPDH) were diluted at 1/1000 and 1/10 000 respectively and incubated 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 paraffinembedded sections) - Anti-NAK/TBK1 antibody
[EPR2867(2)-19] (ab109735)

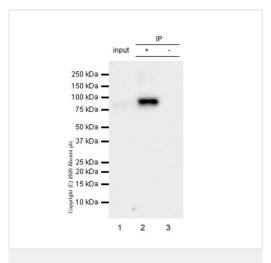
Immunohistochemical analysis of paraffin-embedded human testis tissue using ab109735 at a dilution of 1/250.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-NAK/TBK1 antibody [EPR2867(2)-19] (ab109735)

Immunoprecipitation of TBK1 in HeLa cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of ab109735 pre-coupled to prot.A-Sepharose beads. Samples were washed and processed for western blot with ab12116 at 1 in 2000. This data was kindly provided by the YCharOS Inc., an open science company with the mission of characterizing every commercially available antibody reagent. Abcam are working with YCharOS to support their mission of antibody characterisation using knock out cell lines.



Immunoprecipitation - Anti-NAK/TBK1 antibody [EPR2867(2)-19] (ab109735)

Purified ab109735 at 1/30 dilution ($2\mu g$) immunoprecipitating NAK/TBK1 in HeLa whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate $10\mu g$

Lane 2 (+): ab109735 + HeLa whole cell lysate.

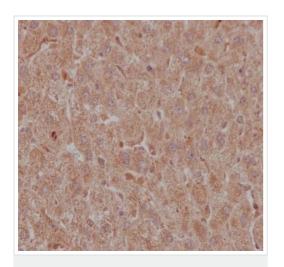
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab109735 in HeLa whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/5000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

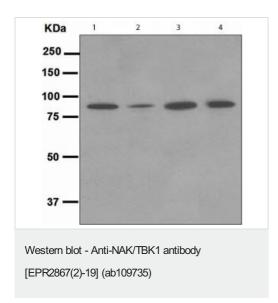
Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 84 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NAK/TBK1 antibody
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ab109735 staining NAK/TBK1 in Human liver tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed and paraffin-embedded, antigen retrieval was by heat mediation in Tris/EDTA buffer pH9. Samples were incubated with primary antibody (1/800). An undiluted HRP-conjugated anti-rabbit IgG was used as the secondary antibody. Tissue counterstained with Hematoxylin.

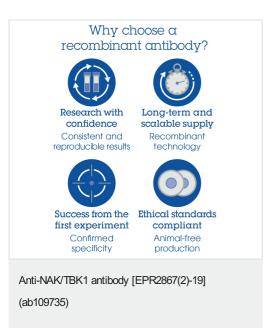


All lanes : Anti-NAK/TBK1 antibody [EPR2867(2)-19] (ab109735) at 1/1000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : U937 cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : 293T cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 84 kDa



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