abcam

Product datasheet

Anti-ITCH/AIP4 antibody [EPR4936] ab108515

KO VALIDATED Recombinant RabMAb

6 References 5 Images

Overview

Product name	Anti-ITCH/AIP4 antibody [EPR4936]
Description	Rabbit monoclonal [EPR4936] to ITCH/AIP4
Host species	Rabbit
Tested applications	**Suitable for:** WB **Unsuitable for:** Flow Cyt,ICC/IF,IHC-P or IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: K562, HeLa, HAP1 and 293T cell lysates. Rat and Mouse brain tissue lysates.
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: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Tissue culture supernatant
Clonality	Monoclonal
Clone number	EPR4936
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab108515 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		1/1000 - 1/10000. Detects a band of approximately 103 kDa (predicted molecular weight: 103 kDa).

Application notes Is unsuitable for Flow Cyt,ICC/IF,IHC-P or IP.

Target

Function Acts as an E3 ubiquitin-protein ligase which accepts ubiquitin from an E2 ubiquitin-conjugating enzyme in the form of a thioester and then directly transfers the ubiquitin to targeted substrates. It catalyzes 'Lys-29'-, 'Lys-48'- and 'Lys-63'-linked ubiquitin conjugation. It is involved in the control of inflammatory signaling pathways. Is an essential component of a ubiquitin-editing protein complex, comprising also TNFAIP3, TAX1BP1 and RNF11, that ensures the transient nature of inflammatory signaling pathways. Promotes the association of the complex after TNF stimulation. Once the complex is formed, TNFAIP3 deubiquitinates 'Lys-63' polyubiquitin chains on RIPK1 and catalyzes the formation of 'Lys-48'-polyubiquitin chains. This leads to RIPK1 proteosomal degradation and consequently termination of the TNF- or LPS-mediated activation of NFKB1. Ubiquitinates RIPK2 by 'Lys-63'-linked conjugation and influences NOD2-dependent signal transduction pathways. Regulates the transcriptional activity of several transcription factors, and probably plays an important role in the regulation of immune response. Ubiquitinates NFE2 by 'Lys-63' linkages and is implicated in the control of the development of hematopoietic lineages. Critical regulator of T helper (TH2) cytokine development through its ability to induce JUNB ubiquitination and degradation (By similarity). Ubiquitinates SNX9. Ubiquitinates CXCR4 and HGS/HRS and regulates sorting of CXCR4 to the degradative pathway. It is involved in the negative regulation of MAVS-dependent cellular antiviral responses. Ubiquitinates MAVS through 'Lys-48'-linked conjugation resulting in MAVS proteosomal degradation. Involved in the regulation of apoptosis and reactive oxygen species levels through the ubiquitination and proteosomal degradation of TXNIP. Mediates the antiapoptotic activity of epidermal growth factor through the ubiquitination and proteosomal degradation of p15 BID. Targets DTX1 for lysosomal degradation and controls NOTCH1 degradation, in the absence of ligand, through 'Lys-29'-linked polyubiquitination.

Tissue specificity Widely expressed.

Pathway Protein modification; protein ubiquitination.

Involvement in disease Defects in ITCH are the cause of syndromic multisystem autoimmune disease (SMAD) [MIM:613385]. SMAD is characterized by organomegaly, failure to thrive, developmental delay, dysmorphic features and autoimmune inflammatory cell infiltration of the lungs, liver and gut.

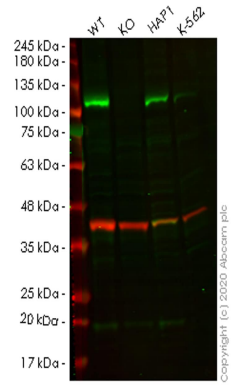
Sequence similarities Contains 1 C2 domain.
Contains 1 HECT (E6AP-type E3 ubiquitin-protein ligase) domain.
Contains 4 WW domains.

Post-translational modifications On T-cell activation, phosphorylation by the JNK cascade on serine and threonine residues surrounding the PRR domain accelerates the ubiquitination and degradation of JUN and JUNB. The increased ITCH catalytic activity due to phosphorylation by JNK1 may occur due to a

conformational change disrupting the interaction between the PRR/WW motifs domain and the HECT domain and, thus exposing the HECT domain (By similarity). Phosphorylation by FYN reduces interaction with JUNB and negatively controls JUN ubiquitination and degradation. Ubiquitinated; autopolyubiquitination with 'Lys-63' linkages which does not lead to protein degradation.

Cellular localization Cell membrane. Cytoplasm. Nucleus. Associates with endocytic vesicles. May be recruited to exosomes by NDFIP1.

Images



Western blot - Anti-ITCH/AIP4 antibody [EPR4936] (ab108515)

All lanes : Anti-ITCH/AIP4 antibody [EPR4936] (ab108515) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ITCH knockout HeLa cell lysate
Lane 3 : HAP1 cell lysate
Lane 4 : K-562 cell lysate

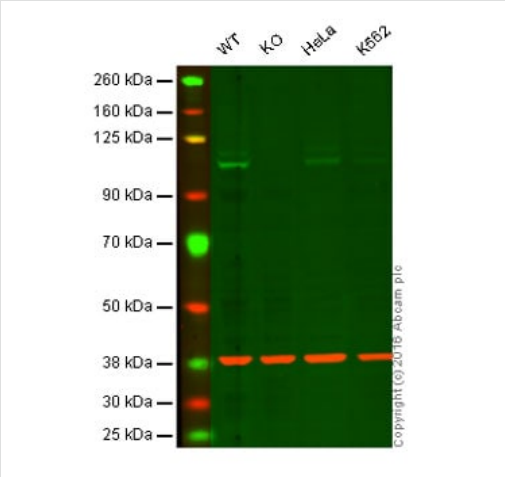
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 103 kDa
Observed band size: 103 kDa

Lanes 1-4: Merged signal (red and green). Green - ab108515 observed at 103 kDa. Red - loading control **ab8245** observed at 36 kDa.

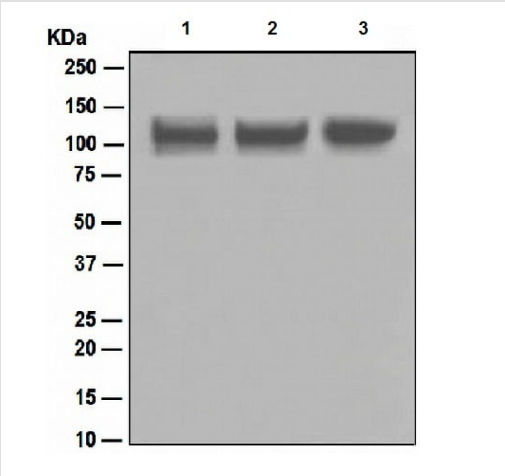
ab108515 Anti-ITCH/AIP4 antibody [EPR4936] was shown to specifically react with ITCH/AIP4 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265338** (knockout cell lysate **ab258014**) was used. Wild-type and ITCH/AIP4 knockout samples were subjected to SDS-PAGE. ab108515 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-ITCH/AIP4 antibody [EPR4936] (ab108515)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: ITCH/AIP4 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: K562 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab108515 observed at 110 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab108515 was shown to specifically react with ITCH/AIP4 when ITCH/AIP4 knockout samples were used. Wild-type and ITCH/AIP4 knockout samples were subjected to SDS-PAGE. ab108515 and **ab8245** (loading control to GAPDH) were diluted 1/500 and 1/10000 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 hour at room temperature before imaging.



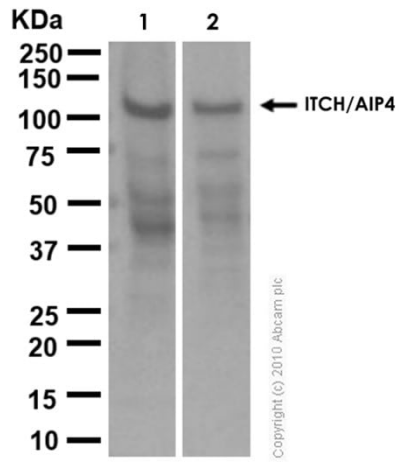
Western blot - Anti-ITCH/AIP4 antibody [EPR4936] (ab108515)

All lanes : Anti-ITCH/AIP4 antibody [EPR4936] (ab108515) at 1/1000 dilution

Lane 1 : K562 cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : 293T cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 103 kDa



Western blot - Anti-ITCH/AIP4 antibody [EPR4936] (ab108515)

All lanes : Anti-ITCH/AIP4 antibody [EPR4936] (ab108515) at 1/1000 dilution

Lane 1 : Mouse brain lysate

Lane 2 : Rat brain 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: 103 kDa

Observed band size: 103 kDa

Exposure time: 1 minute

Blocking buffer and concentration: 5% NFDM/TBST

Anti-ITCH/AIP4 antibody [EPR4936] (ab108515)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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