abcam

Product datasheet

Anti-ITCH/AIP4 antibody [EPR4936] - BSA and Azide free ab247678

KO VALIDATED Recombinant RabMAb

3 Images

Overview

Product name	Anti-ITCH/AIP4 antibody [EPR4936] - BSA and Azide free
Description	Rabbit monoclonal [EPR4936] to ITCH/AIP4 - BSA and Azide free
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	ab247678 is the carrier-free version of **ab108515**. 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. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Affinity purified
Clonality	Monoclonal
Clone number	EPR4936
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab247678 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		Use at an assay dependent concentration. Detects a band of approximately 103 kDa (predicted molecular weight: 103 kDa).

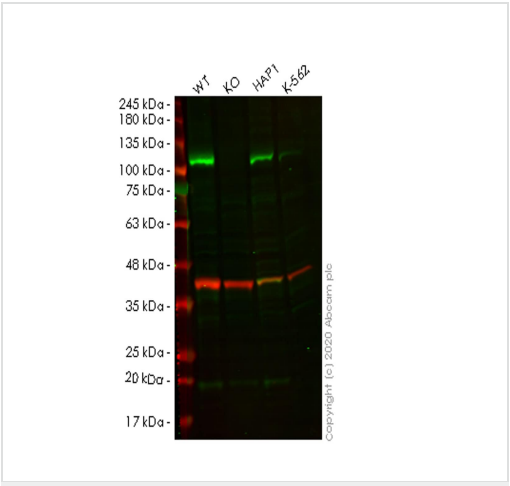
Application notes Is unsuitable for Flow Cyt,ICC/IF,IHC-P or IP.

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] - BSA and Azide free (ab247678)

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.

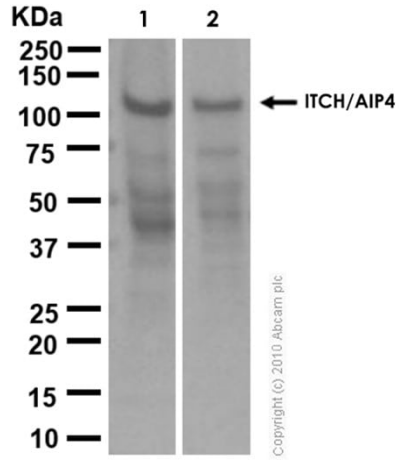
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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

This data was developed using the same antibody clone in a different buffer formulation (**ab108515**).

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] - BSA and Azide free (ab247678)

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.

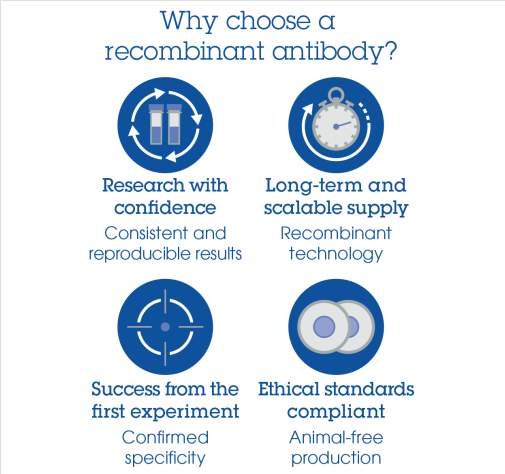
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

Exposure time: 1 minute

This data was developed using **ab108515**, the same antibody clone in a different buffer formulation.

Blocking buffer and concentration: 5% NFDM/TBST



Anti-ITCH/AIP4 antibody [EPR4936] - BSA and Azide free (ab247678)

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