abcam

Product datasheet

Human ITCH (AIP4) knockout HeLa cell lysate ab258014

2 Images

Overview

Product name	Human ITCH (AIP4) knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon7.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab262281 - Human ITCH knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial

Disease Adenocarcinoma

Gender Female

STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

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.

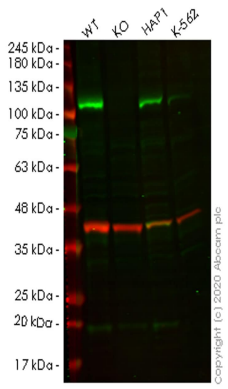
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab258014 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. Predicted molecular weight: 103 kDa.

Images



Western blot - Human ITCH knockout HeLa cell lysate (ab258014)

Lane 1: Wild-type HeLa cell lysate (20 ug)
Lane 2: ITCH knockout HeLa cell lysate (20 ug)
Lane 3: HAP1 cell lysate (20 ug)
Lane 4: K-562 cell lysate (20 ug)

ab108515 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^{o}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.

Sanger Sequencing - Human ITCH knockout HeLa cell lysate (ab258014)

Homozygous: 1 bp insertion in exon7

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