10320 Camino Santa Fe, Suite G
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Anti-ITCH/AIP4 Antibody Picoband™ (monoclonal, 5E12)

Catalog # ABO15099

Specification

Anti-ITCH/AIP4 Antibody Picoband™ (monoclonal, 5E12) - Product Information

Application	**WB, IHC, FC**
Primary Accession	Q96J02
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-ITCH/AIP4 Antibody Picoband™ (monoclonal, 5E12) . Tested in FCM, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Anti-ITCH/AIP4 Antibody Picoband™ (monoclonal, 5E12) - Additional Information

Gene ID 83737

Other Names
E3 ubiquitin-protein ligase Itchy homolog, Itch, 2.3.2.26, Atrophin-1-interacting protein 4, AIP4, HECT-type E3 ubiquitin transferase Itchy homolog, NFE2-associated polypeptide 1, NAPP1, ITCH

Calculated MW
103 kDa KDa

Application Details
Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat
 Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x^6 cells, Human

Contents
Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.

Immunogen
E.coli-derived human ITCH/AIP4 recombinant protein (Position: K17-E358).

Purification
Immunogen affinity purified.

Storage	**At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.**

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Anti-ITCH/AIP4 Antibody Picoband™ (monoclonal, 5E12) - Protein Information

Name ITCH

Function
Acts as an 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 (PubMed:11046148, PubMed:14602072, PubMed:15051726, PubMed:16387660, PubMed:17028573, PubMed:18718448, PubMed:18718449, PubMed:19116316, PubMed:19592251, PubMed:19881509, PubMed:20068034, PubMed:20392206, PubMed:20491914, PubMed:23146885, PubMed:24790097, PubMed:25631046). Catalyzes 'Lys-29'-, 'Lys-48'- and 'Lys-63'-linked ubiquitin conjugation (PubMed:17028573, PubMed:18718448, PubMed:19131965, PubMed:19881509). Involved in the control of inflammatory signaling pathways (PubMed:19131965). Essential component of a ubiquitin-editing protein complex, comprising also TNFAIP3, TAX1BP1 and RNF11, that ensures the transient nature of inflammatory signaling pathways (PubMed:19131965). Promotes the association of the complex after TNF stimulation (PubMed:19131965). Once the complex is formed, TNFAIP3 deubiquitinates 'Lys-63' polyubiquitin chains on RIPK1 and catalyzes the formation of 'Lys-48'-polyubiquitin chains (PubMed:19131965). This leads to RIPK1 proteasomal degradation and consequently termination of the TNF- or LPS-mediated activation of NFKB1 (PubMed:19131965). Ubiquitinates RIPK2 by 'Lys-63'-linked conjugation and influences NOD2-dependent signal transduction pathways (PubMed:19592251). Regulates the transcriptional activity of several transcription factors, and probably plays an important role in the regulation of immune response (PubMed:18718448, PubMed:20491914). Ubiquitinates NFE2 by 'Lys-63' linkages and is implicated in the control of the development of hematopoietic lineages (PubMed:18718448). Mediates JUN ubiquitination and degradation (By similarity). Mediates JUNB ubiquitination and degradation (PubMed:16387660). Critical regulator of type 2 helper T (Th2) cell cytokine production by inducing JUNB ubiquitination and degradation (By similarity). Involved in the negative regulation of MAVS-dependent cellular antiviral responses (PubMed:19881509). Ubiquitinates MAVS through 'Lys-48'-linked conjugation resulting in MAVS proteasomal degradation (PubMed:19881509). Following

ligand stimulation, regulates sorting of Wnt receptor FZD4 to the degradative endocytic pathway probably by modulating PI42KA activity (PubMed:23146885). Ubiquitinates PI4K2A and negatively regulates its catalytic activity (PubMed:23146885). Ubiquitinates chemokine receptor CXCR4 and regulates sorting of CXCR4 to the degradative endocytic pathway following ligand stimulation by ubiquitinating endosomal sorting complex required for transport ESCRT-0 components HGS and STAM (PubMed:14602072, PubMed:23146885, PubMed:34927784). Targets DTX1 for lysosomal degradation and controls NOTCH1 degradation, in the absence of ligand, through 'Lys-29'-linked polyubiquitination (PubMed:17028573, PubMed:18628966, PubMed:23886940). Ubiquitinates SNX9 (PubMed:20491914). Ubiquitinates MAP3K7 through 'Lys-48'-linked conjugation (By similarity). Together with UBR5, involved in the regulation of apoptosis and reactive oxygen species levels through the ubiquitination and proteasomal degradation of TXNIP: catalyzes 'Lys-48'-/'Lys-63'-branched ubiquitination of TXNIP (PubMed:20068034, PubMed:29378950). ITCH synthesizes 'Lys-63'-linked chains, while UBR5 is branching multiple 'Lys-48'-linked chains of substrate initially modified (PubMed:29378950). Mediates the antiapoptotic activity of epidermal growth factor through the ubiquitination and proteasomal degradation of p15 BID (PubMed:20392206). Ubiquitinates BRAT1 and this ubiquitination is enhanced in the presence of NDFIP1 (PubMed:25631046). Inhibits the replication of influenza A virus (IAV) via ubiquitination of IAV matrix protein 1 (M1) through 'Lys-48'-linked conjugation resulting in M1 proteasomal degradation (PubMed:30328013). Ubiquitinates NEDD9/HEF1, resulting in proteasomal degradation of NEDD9/HEF1 (PubMed:15051726).

Cellular Location
Cell membrane; Peripheral membrane protein; Cytoplasmic side. Cytoplasm. Nucleus Early endosome membrane; Peripheral membrane protein; Cytoplasmic side. Endosome membrane; Peripheral membrane protein; Cytoplasmic side. Note=May be recruited to exosomes by NDFIP1 (PubMed:18819914). Localizes to plasma membrane upon CXCL12 stimulation where it co-localizes with CXCL4 (PubMed:14602072) Localization to early endosomes is increased upon CXCL12 stimulation where it co-localizes with DTX3L and CXCL4 (PubMed:24790097)

Tissue Location
Widely expressed.

Anti-ITCH/AIP4 Antibody Picoband™ (monoclonal, 5E12) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry

- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-ITCH/AIP4 Antibody Picoband™ (monoclonal, 5E12) - Images

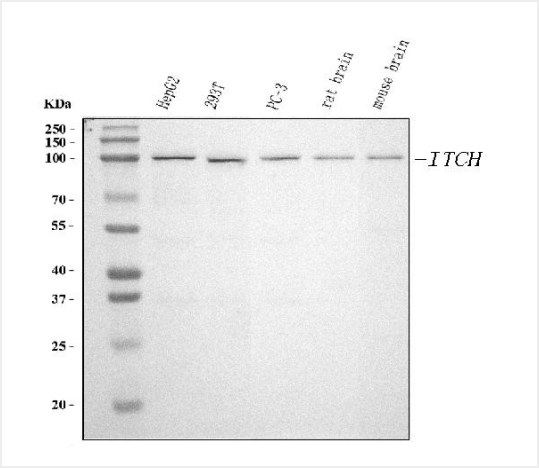

Figure 1. Western blot analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody (M00195-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human HepG2 whole cell lysates,
Lane 2: human 293T whole cell lysates,
Lane 3: human PC-3 whole cell lysates,
Lane 4: rat brain tissue lysates,
Lane 5: mouse brain tissue lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-ITCH/AIP4 antigen affinity purified monoclonal antibody (Catalog # M00195-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for ITCH/AIP4 at approximately 103 kDa. The expected band size for ITCH/AIP4 is at 103 kDa.

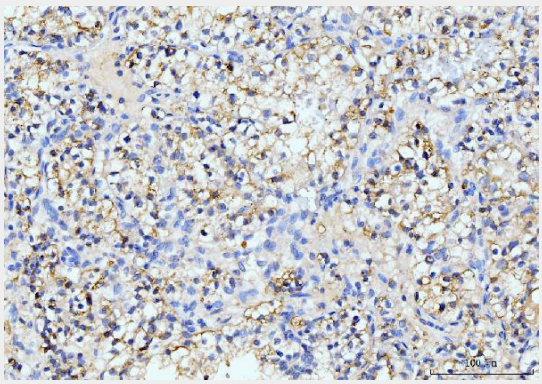

Figure 2. IHC analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody (M00195-1).
ITCH/AIP4 was detected in a paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval

solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-ITCH/AIP4 Antibody (M00195-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

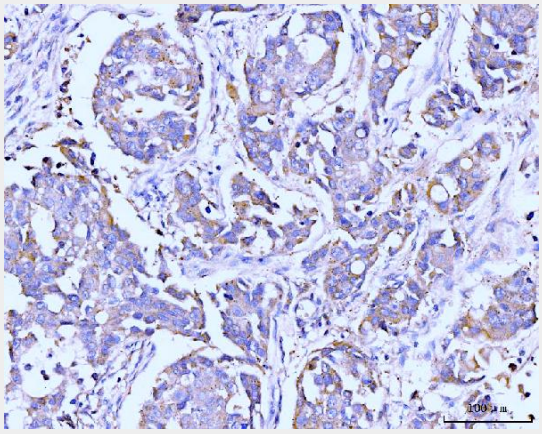

Figure 3. IHC analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody (M00195-1).
ITCH/AIP4 was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-ITCH/AIP4 Antibody (M00195-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

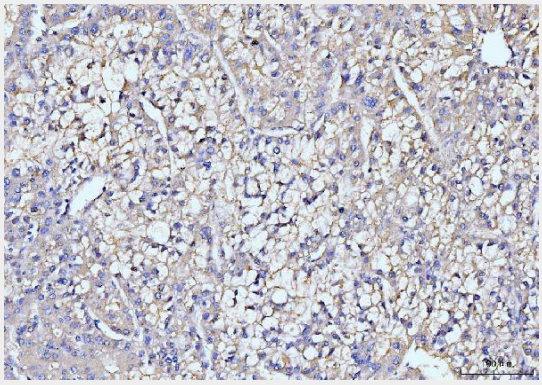

Figure 4. IHC analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody (M00195-1).
ITCH/AIP4 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-ITCH/AIP4 Antibody (M00195-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

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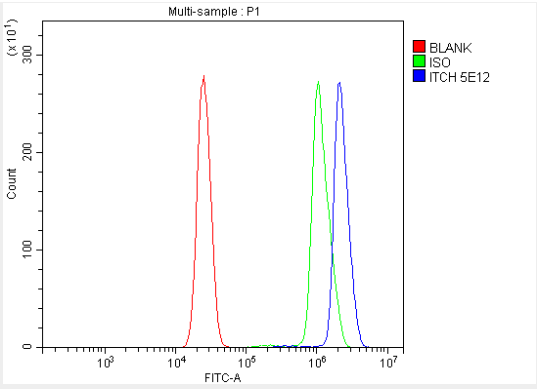

Figure 5. Flow Cytometry analysis of U937 cells using anti-ITCH/AIP4 antibody (M00195-1). Overlay histogram showing U937 cells stained with M00195-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-ITCH/AIP4 Antibody (M00195-1, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Anti-ITCH/AIP4 Antibody Picoband™ (monoclonal, 5E12) - Background

ITCH is an ubiquitin-conjugating enzyme. This gene encodes a member of the Nedd4 family of HECT domain E3 ubiquitin ligases. HECT domain E3 ubiquitin ligases transfer ubiquitin from E2 ubiquitin-conjugating enzymes to protein substrates, thus targeting specific proteins for lysosomal degradation. The encoded protein plays a role in multiple cellular processes including erythroid and lymphoid cell differentiation and the regulation of immune responses. Mutations in this gene are a cause of syndromic multisystem autoimmune disease. Alternatively spliced transcript variants encoding multiple isoforms have been observed for this gene.