

## Anti-ITCH/AIP4 Antibody Picoband®

Catalog Number: A00195-1

### About ITCH

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.

### Overview

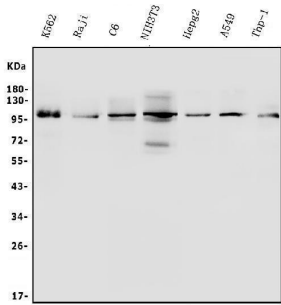
Product Name	Anti-ITCH/AIP4 Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-ITCH/AIP4 Antibody Picoband® catalog # A00195-1. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.01mg NaN <sub>3</sub> .
Storage Instructions	Store 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 freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q96J02

### Technical Details

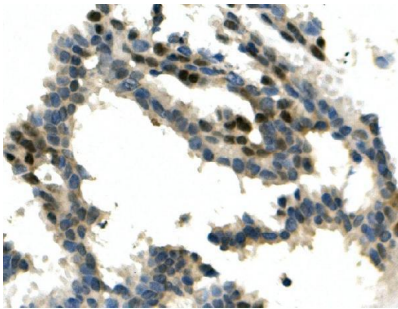
Immunogen	E.coli-derived human ITCH/AIP4 recombinant protein (Position: K17-E358).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.

Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.25ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 2ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10 <sup>6</sup> cells, Human ELISA, 0.1-0.5ug/ml, -

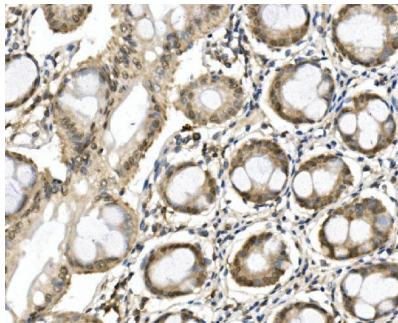
## Anti-ITCH/AIP4 Antibody Picoband® (A00195-1) Images



Western blot analysis of ITCH using anti-ITCH antibody (A00195-1). 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 50ug of sample under reducing conditions. Lane 1: human K562 whole cell lysates, Lane 2: human Raji whole cell lysates, Lane 3: rat C6 whole cell lysates, Lane 4: mouse NIH/3T3 whole cell lysates, Lane 5: human HepG2 whole cell lysates, Lane 6: human A549 whole cell lysates, Lane 7: human THP-1 whole cell lysates. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-ITCH antigen affinity purified polyclonal antibody (Catalog # A00195-1) at 0.25 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit 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 # EK1002) with Tanon 5200 system. A specific band was detected for ITCH at approximately 103KD. The expected band size for ITCH is at 103KD.

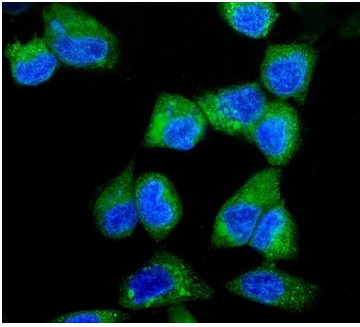


IHC analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody (A00195-1). ITCH/AIP4 was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-ITCH/AIP4 Antibody (A00195-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

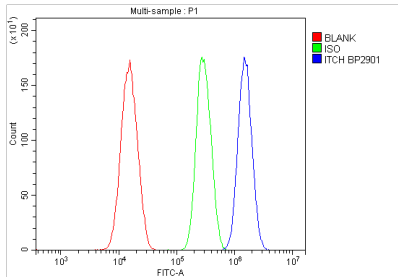


IHC analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody (A00195-1). ITCH/AIP4 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-ITCH/AIP4 Antibody (A00195-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

IF analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody (A00195-1). ITCH/AIP4 was detected in immunocytochemical



section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-ITCH/AIP4 Antibody (A00195-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



Flow Cytometry analysis of SiHa cells using anti-ITCH/AIP4 antibody (A00195-1). Overlay histogram showing SiHa cells stained with A00195-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ITCH/AIP4 Antibody (A00195-1, 1ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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### Anti-ITCH/AIP4 Antibody

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