Product Datasheet

Anti-ITCH/AIP4 Antibody (monoclonal, 5E12) (orb865595)

Catalog Number	orb865595
Description	Anti-ITCH/AIP4 Antibody (monoclonal, 5E12). Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Species/Host	Mouse
Reactivity	Human, Mouse, Rat
Conjugation	Unconjugated
Tested Applications	FC, IHC, WB
Immunogen	E.coli-derived human ITCH/AIP4 recombinant protein (Position: K17-E358).
Form/Appearance	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Note	For research use only
Application notes	Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human Flow Cytometry (Fixed), 1-3 µg/1x6 cells, Human. Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml
Isotype	Mouse IgG2b
Clonality	Monoclonal
Clone Number	5E12

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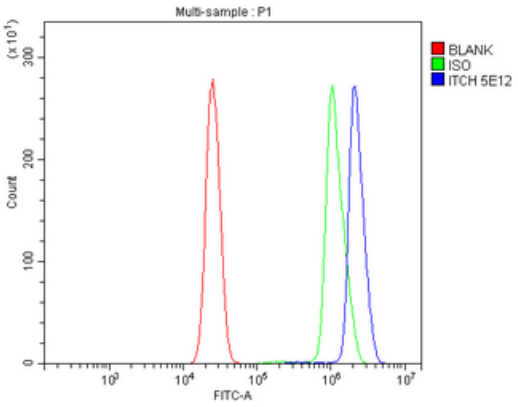
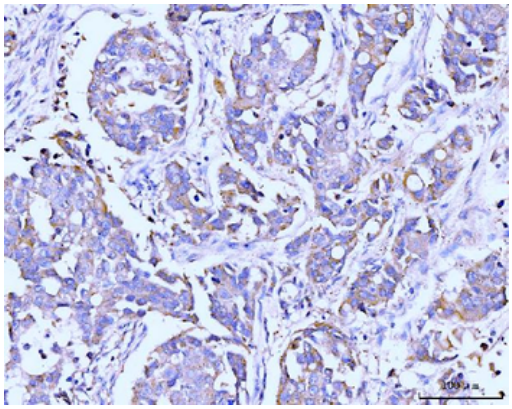
Antibody Type	Primary Antibody
MW	103 kDa
Uniprot ID	**Q96J02**
Expiration Date	12 months from date of receipt.



Flow Cytometry analysis of U937 cells using anti-ITCH/AIP4 antibody. Overlay histogram showing U937 cells (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 mouse anti-ITCH/AIP4 Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (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 without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



IHC analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody. ITCH/AIP4 was detected in a paraffin-embedded section of human breast 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 2 µg/ml mouse anti-ITCH/AIP4 Antibody 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) with DAB as the chromogen.

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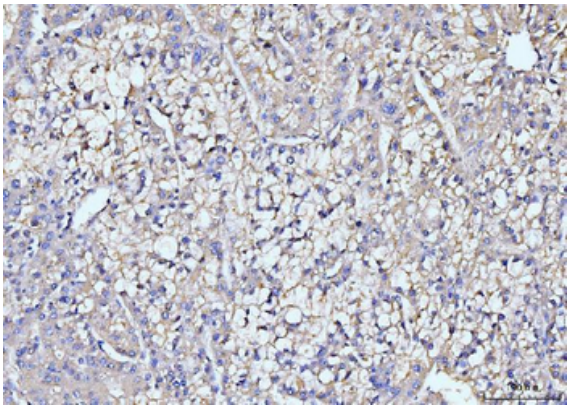
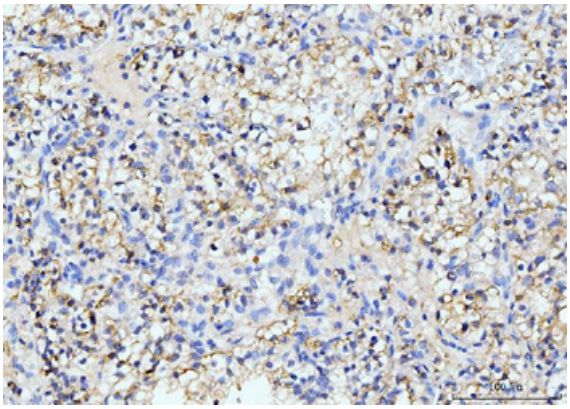


IHC analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody. ITCH/AIP4 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-ITCH/AIP4 Antibody 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) with DAB as the chromogen.



IHC analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody. 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 (pH8.0, 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 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) with DAB as the chromogen.

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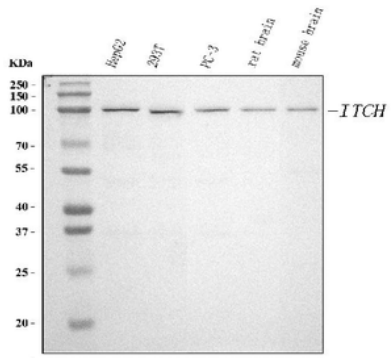

Western blot analysis of ITCH/AIP4 using anti-ITCH/AIP4 antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human HepG2 whole cell lysates, 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 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 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.

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