10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Anti-STUB1 Antibody Picoband™ (monoclonal, 13I8)

Catalog # ABO14848

Specification

Anti-STUB1 Antibody Picoband™ (monoclonal, 13I8) - Product Information

Application	**WB, IHC, FC**
Primary Accession	Q9UNE7
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse, Monkey**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-STUB1 Antibody Picoband™ (monoclonal, 13I8) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.

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

Anti-STUB1 Antibody Picoband™ (monoclonal, 13I8) - Additional Information

Gene ID 10273

Other Names
E3 ubiquitin-protein ligase CHIP, 2.3.2.27, Antigen NY-CO-7, CLL-associated antigen KW-8, Carboxy terminus of Hsp70-interacting protein, RING-type E3 ubiquitin transferase CHIP, STIP1 homology and U box-containing protein 1 {ECO:0000312|HGNC:HGNC:11427}, STUB1 {ECO:0000303|PubMed:23973223, ECO:0000312|HGNC:HGNC:11427}

Calculated MW
35 kDa KDa

Application Details
Western blot, 0.1-0.5 µg/ml
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml
 Flow Cytometry, 1-3 µg/1x10^6 cells

Subcellular Localization
Nucleus. Cytoplasm.

Tissue Specificity
Expressed in differentiated myotubes (at protein level). Highly expressed in skeletal muscle, heart, pancreas, brain and placenta. Detected in kidney, liver and lung.

Contents
E. coli-derived human STUB1 recombinant protein (Position: R51-Y303). Human STUB1 shares 98% amino acid (aa) sequence identity with both mouse and rat STUB1.

Immunogen
E.coli-derived human STUB1 recombinant protein (Position: R51-Y303).

Cross Reactivity
No cross-reactivity with other proteins.

Storage

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.

Anti-STUB1 Antibody Picoband™ (monoclonal, 13I8) - Protein Information

Name STUB1 {ECO:0000303|PubMed:23973223, ECO:0000312|HGNC:HGNC:11427}

Function
E3 ubiquitin-protein ligase which targets misfolded chaperone substrates towards proteasomal degradation (PubMed:10330192, PubMed:11146632, PubMed:11557750, PubMed:23990462, PubMed:26265139). Plays a role in the maintenance of mitochondrial morphology and promotes mitophagic removal of dysfunctional mitochondria; thereby acts as a protector against apoptosis in response to cellular stress (By similarity). Negatively regulates vascular smooth muscle contraction, via degradation of the transcriptional activator MYOCD and subsequent loss of transcription of genes involved in vascular smooth muscle contraction (By similarity). Promotes survival and proliferation of cardiac smooth muscle cells via ubiquitination and degradation of FOXO1, resulting in subsequent repression of FOXO1-mediated transcription of pro-apoptotic genes (PubMed:19483080). Ubiquitinates ICER-type isoforms of CREM and targets them for proteasomal degradation, thereby acts as a positive effector of MAPK/ERK-mediated inhibition of apoptosis in cardiomyocytes (PubMed:20724525). Inhibits lipopolysaccharide-induced apoptosis and hypertrophy in cardiomyocytes, via ubiquitination and subsequent proteasomal degradation of NFATC3 (PubMed:30980393). Collaborates with ATXN3 in the degradation of misfolded chaperone substrates: ATXN3 restricting the length of ubiquitin chain attached to STUB1/CHIP substrates and preventing further chain extension (PubMed:10330192, PubMed:11146632, PubMed:11557750, PubMed:23990462). Ubiquitinates NOS1 in concert with Hsp70 and Hsp40 (PubMed:15466472). Modulates the activity of several chaperone complexes, including Hsp70, Hsc70 and Hsp90 (PubMed:10330192, PubMed:11146632, PubMed:15466472). Ubiquitinates CHRNA3 targeting it for endoplasmic reticulum-associated degradation in cortical neurons, as part of the STUB1-VCP-UBXN2A complex (PubMed:26265139). Ubiquitinates and promotes ESR1 proteasomal degradation in response to age-related circulating estradiol (17-beta-estradiol/E2) decline, thereby promotes neuronal apoptosis in response to ischemic reperfusion injury (By similarity). Mediates transfer of non-canonical short ubiquitin chains to HSPA8 that have no effect on HSPA8 degradation (PubMed:11557750, PubMed:23990462). Mediates polyubiquitination of DNA polymerase beta (POLB) at 'Lys-41', 'Lys-61' and 'Lys-81', thereby

playing a role in base-excision repair: catalyzes polyubiquitination by amplifying the HUWE1/ARF-BP1-dependent monoubiquitination and leading to POLB-degradation by the proteasome (PubMed:19713937). Mediates polyubiquitination of CYP3A4 (PubMed:19103148). Ubiquitinates EPHA2 and may regulate the receptor stability and activity through proteasomal degradation (PubMed:19567782). Acts as a co-chaperone for HSPA1A and HSPA1B chaperone proteins and promotes ubiquitin-mediated protein degradation (PubMed:27708256). Negatively regulates the suppressive function of regulatory T-cells (Treg) during inflammation by mediating the ubiquitination and degradation of FOXP3 in a HSPA1A/B-dependent manner (PubMed:23973223). Catalyzes monoubiquitination of SIRT6, preventing its degradation by the proteasome (PubMed:24043303). Likely mediates polyubiquitination and down-regulates plasma membrane expression of PD-L1/CD274, an immune inhibitory ligand critical for immune tolerance to self and antitumor immunity (PubMed:28813410). Negatively regulates TGF-beta signaling by modulating the basal level of SMAD3 via ubiquitin-mediated degradation (PubMed:24613385). Plays a role in the degradation of TP53 (PubMed:26634371). Mediates ubiquitination of RIPK3 leading to its subsequent proteasome-dependent degradation (PubMed:29883609). May regulate myosin assembly in striated muscles together with UBE4B and VCP/p97 by targeting myosin chaperone UNC45B for proteasomal degradation (PubMed:17369820). Ubiquitinates PPARG in macrophages playing a role in M2 macrophages polarization and angiogenesis (By similarity).

Cellular Location
Cytoplasm. Nucleus. Mitochondrion {ECO:0000250|UniProtKB:A6HD62}. Note=Translocates to the nucleus in response to inflammatory signals in regulatory T-cells (Treg) Localizes to mitochondria following oxygen and glucose deprivation- induced cellular stress (By similarity). {ECO:0000250|UniProtKB:A6HD62, ECO:0000269|PubMed:23973223}

Tissue Location
Expressed in differentiated myotubes (at protein level) (PubMed:17369820). Highly expressed in skeletal muscle, heart, pancreas, brain and placenta (PubMed:10330192, PubMed:11435423) Detected in kidney, liver and lung (PubMed:10330192, PubMed:11435423)

Anti-STUB1 Antibody Picoband™ (monoclonal, 13I8) - 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-STUB1 Antibody Picoband™ (monoclonal, 13I8) - Images

10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

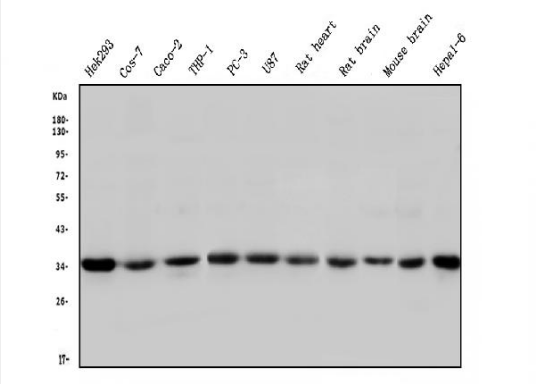

Figure 1. Western blot analysis of STUB1 using anti-STUB1 antibody (M01236).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human HEK293 whole cell lysates,
Lane 2: monkey COS-7 whole cell lysates,
Lane 3: human CACO-2 whole cell lysates.
Lane 4: human THP-1 whole cell lysates,
Lane 5: human PC-3 whole cell lysates,
Lane 6: human U87 whole cell lysates,
Lane 7: rat heart tissue lysates,
Lane 8: rat brain tissue lysates,
Lane 9: mouse brain tissue lysates,
Lane 10: mouse HEPA1-6 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 mouse anti-STUB1 antigen affinity purified monoclonal antibody (Catalog # M01236) 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 STUB1 at approximately 35KD. The expected band size for STUB1 is at 35KD.

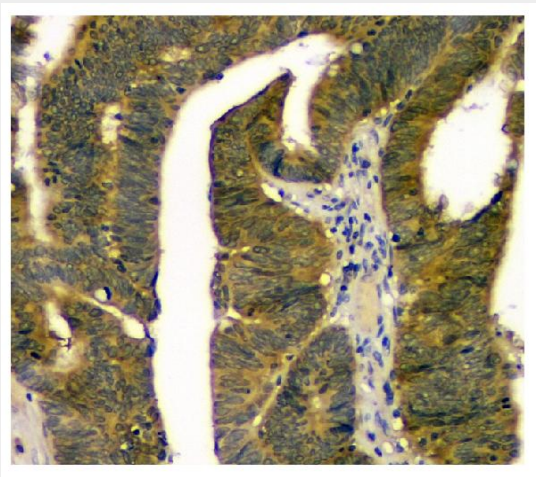

Figure 2. IHC analysis of STUB1 using anti STUB1 antibody (M01236).
STUB1 was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue

section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-STUB1 Antibody (M01236) 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.

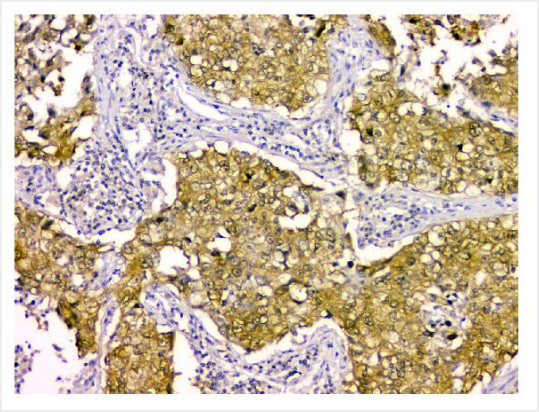

Figure 3. IHC analysis of STUB1 using anti STUB1 antibody (M01236).
STUB1 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-STUB1 Antibody (M01236) 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.

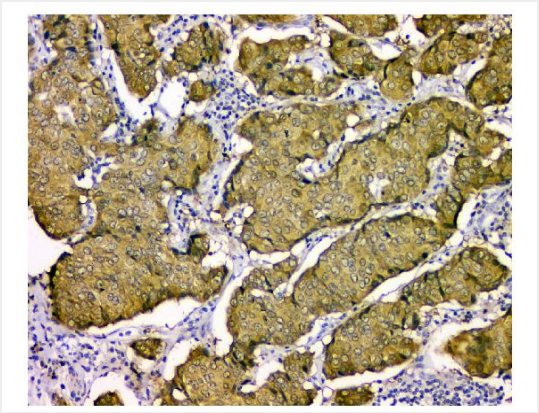

Figure 4. IHC analysis of STUB1 using anti STUB1 antibody (M01236).
STUB1 was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-STUB1 Antibody (M01236) 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.

10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

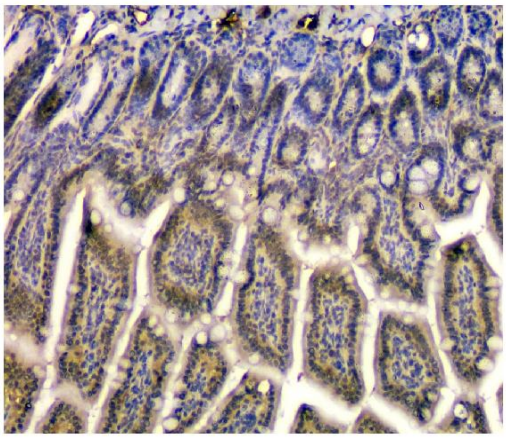

Figure 5. IHC analysis of STUB1 using anti STUB1 antibody (M01236).
STUB1 was detected in paraffin-embedded section of mouse small intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-STUB1 Antibody (M01236) 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.

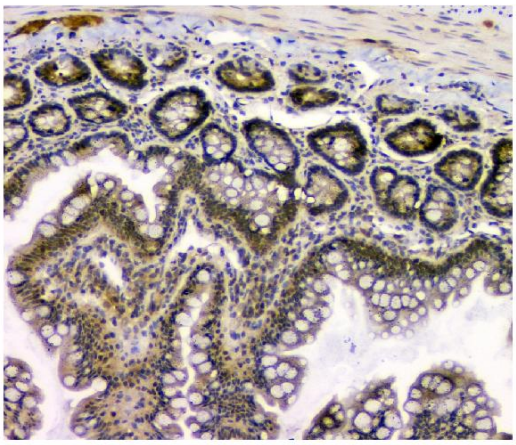

Figure 6. IHC analysis of STUB1 using anti STUB1 antibody (M01236).
STUB1 was detected in paraffin-embedded section of rat small intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-STUB1 Antibody (M01236) 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.

10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

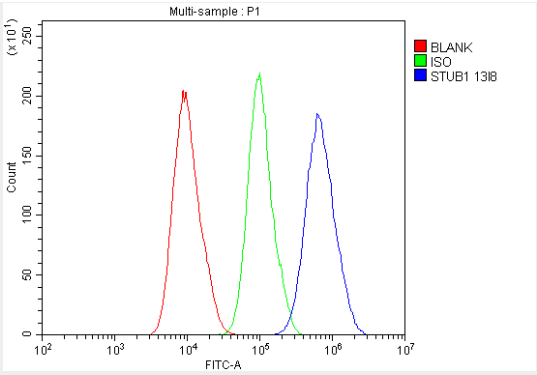

Figure 7. Flow Cytometry analysis of A549 cells using anti-STUB1 antibody (M01236).
Overlay histogram showing A549 cells stained with M01236 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-STUB1 Antibody (M01236, 1 µg/1x10^6 cells) for 30 min at 20°C. 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-STUB1 Antibody Picoband™ (monoclonal, 13I8) - Background

STUB1 (STIP1 homology and U-Box containing protein 1), also known as CHIP (C terminus of HSC70-Interacting Protein), is a human gene. This gene encodes a protein containing tetratricopeptide repeat and a U-box that functions as a ubiquitin ligase/cochaperone. The encoded protein binds to and ubiquitinates shock cognate 71 kDa protein (Hspa8) and DNA polymerase beta (Polb), among other targets. Mutations in this gene cause spinocerebellar ataxia, autosomal recessive 16. Alternative splicing results in multiple transcript variants. There is a pseudogene for this gene on chromosome 2.