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Anti-Tuberin TSC2 Antibody Picoband™ (monoclonal, 6I3)

Catalog # ABO14893

Product Information

Application	WB, IHC, FC
Primary Accession	P49815
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Tuberin TSC2 Antibody Picoband™ (monoclonal, 6I3) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	7249
Other Names	Tuberin, Tuberous sclerosis 2 protein, TSC2 {ECO:0000303|PubMed:7558029, ECO:0000312|HGNC:HGNC:12363}
Calculated MW	200608
Application Details	Western blot, 0.1-0.5 □g/ml, Human Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml, Human, By Heat Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Subcellular Localization	Cytoplasm; Membrane; Peripheral membrane protein
Tissue Specificity	Liver, brain, heart, lymphocytes, fibroblasts, biliary epithelium, pancreas, skeletal muscle, kidney, lung and placenta.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 6I3
Immunogen	E.coli-derived human Tuberin recombinant protein (Position: H1611-V1807). Human Tuberin shares 94% and 90% amino acid (aa) sequence identity with mouse and rat Tuberin, respectively.
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.

Protein Information

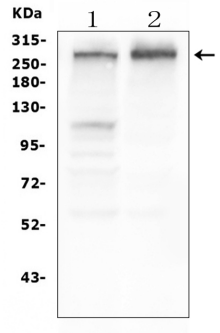
Name	TSC2 {ECO:0000303|PubMed:7558029, ECO:0000312|HGNC:HGNC:12363}
Function	Catalytic component of the TSC-TBC complex, a multiprotein complex that acts as a negative regulator of the canonical mTORC1 complex, an evolutionarily conserved central nutrient sensor that stimulates anabolic reactions and macromolecule biosynthesis to promote cellular biomass generation and growth (PubMed:12172553, PubMed:12271141, PubMed:12842888, PubMed:12906785, PubMed:15340059, PubMed:22819219, PubMed:24529379, PubMed:28215400, PubMed:33436626, PubMed:35772404). Within the TSC-TBC complex, TSC2 acts as a GTPase- activating protein (GAP) for the small GTPase RHEB, a direct activator of the protein kinase activity of mTORC1 (PubMed:12172553, PubMed:12820960, PubMed:12842888, PubMed:12906785, PubMed:15340059, PubMed:22819219, PubMed:24529379, PubMed:33436626). In absence of nutrients, the TSC-TBC complex inhibits mTORC1, thereby preventing phosphorylation of ribosomal protein S6 kinase (RPS6KB1 and RPS6KB2) and EIF4EBP1 (4E-BP1) by the mTORC1 signaling (PubMed:12172553, PubMed:12271141, PubMed:12842888, PubMed:12906785, PubMed:22819219, PubMed:24529379, PubMed:28215400, PubMed:35772404). The TSC-TBC complex is inactivated in response to nutrients, relieving inhibition of mTORC1 (PubMed:12172553, PubMed:24529379). Involved in microtubule-mediated protein transport via its ability to regulate mTORC1 signaling (By similarity). Also stimulates the intrinsic GTPase activity of the Ras- related proteins RAP1A and RAB5 (By similarity).
Cellular Location	Lysosome membrane; Peripheral membrane protein. Cytoplasm, cytosol Note=Recruited to lysosomal membranes in a RHEB-dependent process in absence of nutrients (PubMed:24529379). In response to insulin signaling and phosphorylation by PKB/AKT1, the complex dissociates from lysosomal membranes and relocalizes to the cytosol (PubMed:24529379)
Tissue Location	Liver, brain, heart, lymphocytes, fibroblasts, biliary epithelium, pancreas, skeletal muscle, kidney, lung and placenta.

Background

Tuberous sclerosis 2 protein, also known as TSC2 or Tuberin is a protein that is in humans. The chromosomal location of this gene is 16p13.3. Mutations in this gene lead to tuberous sclerosis complex. Its gene product is believed to be a tumor suppressor and is able to stimulate specific GTPases. The protein associates with hamartin in a cytosolic complex, possibly acting as a chaperone for hamartin. This gene involved in microtubule-mediated protein transport, but this seems to be due to unregulated mTOR signaling. It stimulates weakly the intrinsic GTPase activity of the Ras-related proteins RAP1A and RAB5 in vitro.

Images

Figure 1. Western blot analysis of TSC2 using anti-TSC2 antibody (M00229-1).
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 HEK293 tissue lysates,
Lane 2: human PC-3 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-TSC2 antigen affinity purified polyclonal antibody (Catalog # M00229-1) 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 TSC2 at approximately 280KD. The expected band size for TSC2 is at 201KD.

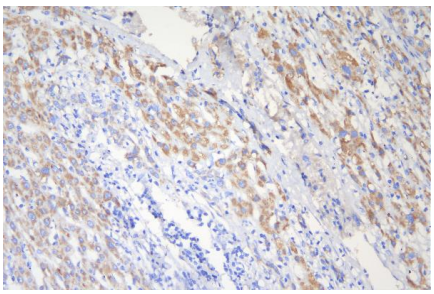


Figure 2. IHC analysis of TSC2 using anti-TSC2 antibody (M00229-1).
TSC2 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-TSC2 Antibody (M00229-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

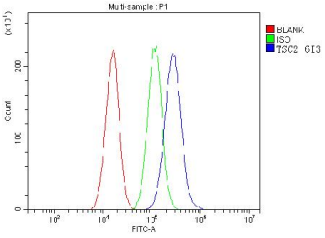


Figure 3. Flow Cytometry analysis of A549 cells using anti-TSC2 antibody (M00229-1).
Overlay histogram showing A549 cells stained with M00229-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-TSC2 Antibody (M00229-1, 1 µg/1x10^6 cells) for 30 min at 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.

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