

Anti-Tuberin TSC2 Antibody Picoband[™] (monoclonal, 6l3)

Catalog # ABO14893

Anti-Tuberin TSC2 Antibody Picoband[™] (monoclonal, 6I3) - Product Information

Application	
Primary Accession	
Host	
Isotype	
Reactivity	
Clonality	
Format	
Description	

WB, IHC, FC P49815 Mouse Mouse IgG2b Human Monoclonal Lyophilized

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 μ g/ml.

Anti-Tuberin TSC2 Antibody Picoband[™] (monoclonal, 613) - Additional Information

Gene ID 7249

Other Names Tuberin, Tuberous sclerosis 2 protein, TSC2 {ECO:0000303|PubMed:7558029, ECO:0000312|HGNC:HGNC:12363}

Calculated MW 280 kDa KDa

Application Details Western blot, 0.1-0.5 μ g/ml, Human
 Immunohistochemistry (Paraffin-embedded Section), 0.5-1 μ g/ml, Human, By Heat
 Flow Cytometry, 1-3 μ 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.

Contents

Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na₂HPO₄, 0.05mg NaN₃.

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.

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

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

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

- <u>Western Blot</u>
- Blocking Peptides
- <u>Dot Blot</u>
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

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

KDa	1	2	
315-			
250-	-	-	-
180-			
130-			
95-			
72-			
52-			
43-			

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 μ g/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 μ g/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 μ g/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 μ g/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 μ g/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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