

Anti-TRPC6 Monoclonal Antibody

Catalog Number: M00625

About TRPC6

TRPC6, also known as TRP6, short transient receptor potential channel 6 and transient receptor potential cation channel subfamily C member 6, is thought to form a receptor-activated non-selective calcium permeant cation channel. TRPC6 is probably operated by a phosphatidylinositol second messenger system activated by receptor tyrosine kinases or G-protein coupled receptors. It is activated by diacylglycerol (DAG) in a membrane-delimited fashion, independently of protein kinase C and may not to be activated by intracellular calcium store depletion. Defects in this gene are a cause of focal segmental glomerulosclerosis (FSGS). Expression of this protein has been reported in tissues such as placenta, lung, spleen, ovary, small intestine, and renal podocytes. Immunohistochemistry studies using polyclonal antibodies to this target have shown moderate to strong staining in cell types such as neurons, breast, respiratory, squamous and prostate epithelium, epidermis, placental trophoblasts, dendritic cells, and subsets of immune cells, and faint to moderate staining of adrenal, colon, ganglion cells, hepatocytes, heart, and testis.

Overview

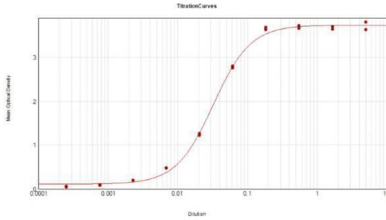
Product Name	Anti-TRPC6 Monoclonal Antibody
Reactive Species	Human, Mouse
Description	Boster Bio Anti-TRPC6 Monoclonal Antibody (Catalog # M00625). Tested in ELISA, IHC, WB applications. This antibody reacts with Human, Mouse.
Application	ELISA, IHC, WB
Clonality	Monoclonal Clone: 3F2.H10.F2 IgG1 kappa
Formulation	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2, 0.01% (w/v) Sodium Azide
Storage Instructions	Store vial at -20°C prior to opening. Aliquot contents and freeze at -20°C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4°C as an undiluted liquid. Dilute only prior to immediate use. Expiration date is one (1) year from date of opening. (Ship on dry ice.)
Host	Mouse
Uniprot ID	Q9Y210

Technical Details

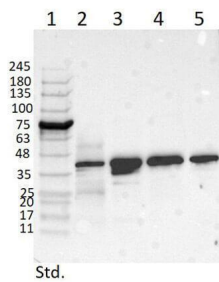
Immunogen	This monoclonal antibody was produced by repeated immunizations with a synthetic peptide corresponding to a region near the carboxy terminus of human TRPC6 protein.
Predicted Reactive Species	Chimpanzee
Cross Reactivity	No cross reactivity with other proteins.
Isotype	IgG1 kappa

Form	Liquid (sterile filtered)
Concentration	0.964 mg/mL by UV absorbance at 280 nm
Purification	This product was purified from concentrated tissue culture supernate by Protein A chromatography. This antibody is specific for human TRPC6 protein. A BLAST analysis was used to suggest cross-reactivity with TRPC6 from chimpanzee based on 100% homology with the immunizing sequence. Cross-reactivity with TRPC6 from other sources has not been determined.
Suggested Dilutions	<p>ELISA: 1:10,000 - 1:50,000 IHC: 2.5 µg/mL WB: 1:500- 1:2,000</p> <p>Anti-TRPC6 monoclonal antibody (200-301-B59) clone # 3F2.H10.F2 was developed by Rockland Immunochemicals Inc. against human TRPC6 using conventional hybridoma technology by fusing splenocytes of a host animal immunized with a synthetic peptide corresponding to the cytosolic domain of TRPC6 with myeloma cells. The screening of clones during the subcloning process was based on immunohistochemistry using human tissue microarrays. The pathologist analyzing the staining patterns of clones reported that the antibody shows strong to moderate staining consistent with the localization of human TRPC6 in adrenal cortex, neurons, Purkinje cells, colon epithelium, cardiac myocytes, renal tubules, hepatocytes, skeletal muscle, pancreatic exocrine and islet cells, germinal center lymphocytes, plasma cells, Sertoli cells of the testes as well as staining more faintly other tissues known to be positive for the target protein (e.g., respiratory epithelium). Prostate and placenta were negative for staining. The antibody produced minimal to no background staining and appeared very specific at 2.5 µg/mL. The pattern of reactivity observed for this clone was also similar to other antibodies used for benchmarking purposes. Specific conditions for reactivity should be optimized by the end user, however, we suggest the use of formalin-fixed paraffin-embedded sections for immunohistochemistry. No pre-treatment of sample is required. While immunohistochemistry was used as the primary screening and release validation immunoassay, clone #3F2.H10.F2 was also screened by western blotting against known positive and negative control lysates. A single band is detected by this antibody in TRPC6 positive cells and tissues; however, the molecular weight of the band (~30 kDa) is not consistent with full length human TRPC6 (181 kDa). The band detected by this antibody may be the cleaved cytosolic domain of TRPC6 as the immunogen used for antibody production corresponds to an amino acid sequence located within this domain. However, no additional data is available to elucidate the molecular composition of this band.</p>

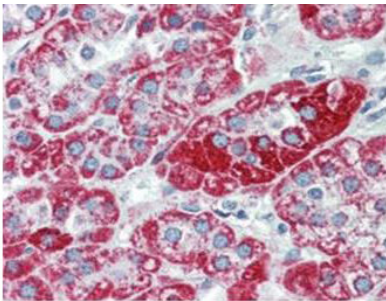
Anti-TRPC6 Monoclonal Antibody (M00625) Images



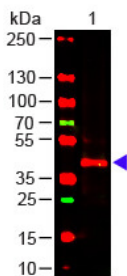
ELISA Results of Mouse Anti-TRPC6 Antibody. Each well was coated in duplicate with 0.1µg of conjugate. The working dilution is 1:31,000. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using HRP conjugation Stabilizer , Rabbit Anti-Mouse IgG HRP conjugated and TMB substrate .



Western Blot of Mouse Anti-TRPC6 Antibody. Lane 1: Opal Prestained Molecular Weight Marker . Lane 2: Mouse Pancreas Tissue Lysate [10µg]. Lane 3: MCF-7 Whole Cell Lysate [10µg]. Lane 4: A431 Whole Cell Lysate [10µg]. Lane 5: Jurkat Whole Cell Lysate [10µg]. Primary Antibody: Anti-TRPC6 at 1µg/mL overnight at 2-8°C. Secondary Antibody: Rabbit Anti-Mouse IgG Peroxidase 1:40000 for 30mins at RT. Blocking Buffer: BlockOut Buffer for 30mins at RT. Predicted MW: ~30kDa. Observed MW: ~40kDa. Exposure: 5sec.



Immunohistochemistry using Boster's anti-TRPC6 monoclonal antibody shows detection of TRPC6 in human adrenal (cortex) tissue (40X). The antibody was used a dilution to 2.5 µg/mL. The image shows strong staining with minimal background staining. Tissue was formalin fixed and paraffin embedded. No pre-treatment of sample was required. The image shows the localization of antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Personal communication, Andrew Elston, Lifespan Biosciences, Seattle, WA.



Western Blot of Mouse anti-TRPC6 Antibody. Lane 1: Mouse Kidney WCL . Load: 10 µg per lane. Primary antibody: TRPC6 Antibody at 1:1000 for overnight at 4°C. Secondary antibody: donkey anti-mouse DyLight™ 649 at 1:20,000 for 30 min at RT. Block: 30 min at RT.

1 Publications Citing This Product

1. PubMed ID: 27472391, TRPC1/TRPC3 channels mediate lysophosphatidylcholine-induced apoptosis in cultured human coronary artery smooth muscles cells

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