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Datasheet for 200-301-B59

TRPC6 Antibody

Overview

Description:	Anti-TRPC6 (MOUSE) Monoclonal Antibody - 200-301-B59
Item No.:	200-301-B59
Size:	100 µg
Applications:	ELISA, IHC, WB
Reactivity:	Human, Mouse
Host Species:	Mouse

Product Details

Background:	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.
Synonyms:	mouse anti-TRPC6 Antibody, TRPC 6, TRP6, short transient receptor potential channel 6 and transient receptor potential cation channel subfamily C member 6
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	3F2.H10.F2
Format:	IgG1

Target Details

Gene Name:	TRPC6
Reactivity:	Human, Mouse
Immunogen Type:	Conjugated Peptide
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.
Purity/Specificity:	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.
Relevant Links:	• UniProtKB - Q9Y210 • NCBI - 5730102 • GeneID - 7225

Application Details

Tested Applications:	ELISA, IHC, WB

Application Note:

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.

Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:50,000
IHC:	2.5 µg/mL
WB:	1:500- 1:2,000

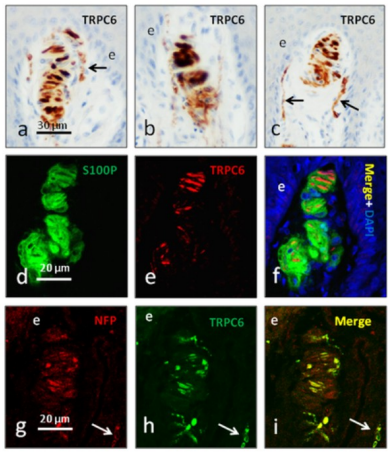
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	0.964 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

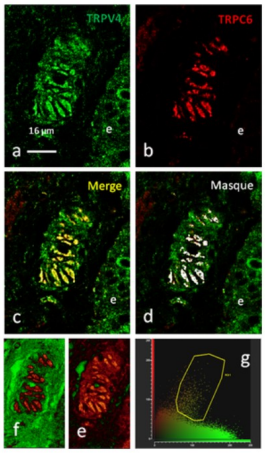
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	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:	Expiration date is one (1) year from date of receipt.

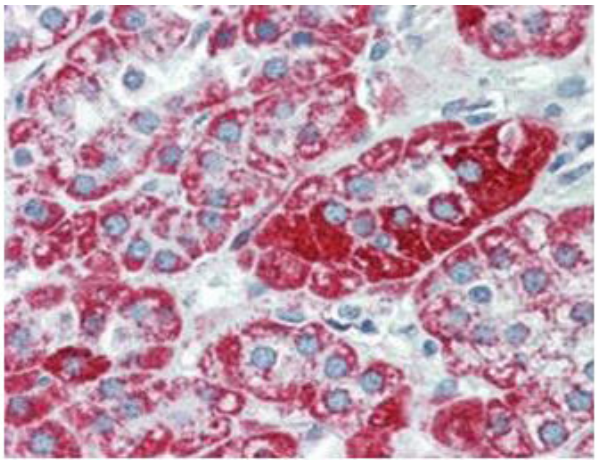
Images



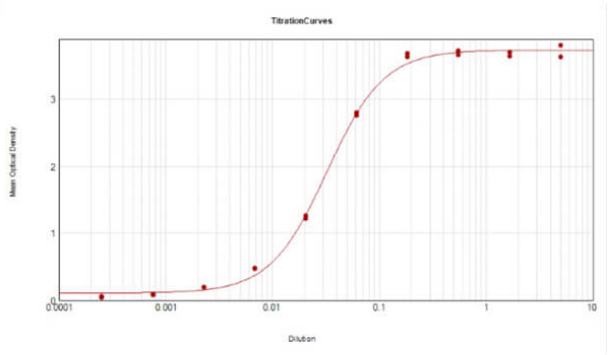
Immunofluorescence Microscopy
Immunohistochemical localization TRPC6 using anti-TRPC6 monoclonal (A,B) and polyclonal (C) antibodies in human digital Meissner corpuscles (around 22%). Immunoreactivity was found in axons supplying Meissner corpuscles and isolated thick nerve fibers (arrows). The pattern of TRPC6 immunolabelling (E and H; red and green fluorescence, respectively), matches the distribution of NFP (G; red fluorescence) but not of S100 protein (D, green fluorescence). When the images were overlapped there was colocalization of TRPC6 and NFP (I, merge) but not colocalization of TRPC6 and S100 protein (F, merge). Objective 40×/1.25 Oil; pinhole airy 1, XY resolution 156 nm and Z resolution 334 nm. e: epidermis.
Fig 1.
PMID: 27874267



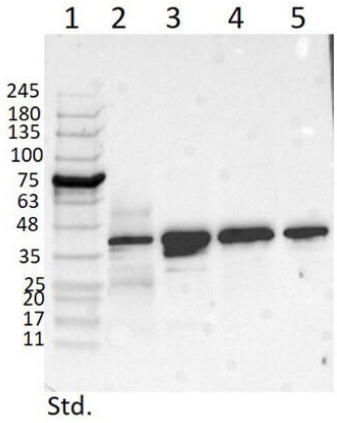
Immunofluorescence Microscopy
Confocal laser-scanning images of TRPV4 (green fluorescence, A) and TRPC6 (red, B) in one single digital Meissner corpuscle. Figure C is a merge image of TRPV4 and TRPC6 covered by mask (D, white dots). Mask represents the regions of interest drawn on the cytofluorogram around the high red signal dots. Images E and F show the quantification of fluorescente intensity of images 3A and 3B, respectively. The values range from 0 (green pixels) to 255 (blue pixels and the absence of blue pixels indicate that the images are unsaturated. Image in g shows a 2D cytofluorogram from the two detection channels from the original image. Every dot of cytofluorogram represents an intensity value pair from the two detection channels. Red signal dots are not close to the Y-axis because the green signal is widely distributed (including epidermis) and has been captured with higher gain amplification than those for the red signal. The area of interest surrounding the high red signal dots on cytofluorogram had been drawn and, on the merge image, is represented as a mask (white dots). e: epidermis.
Fig 4.
PMID: 27874267



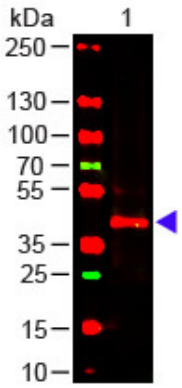
Immunohistochemistry
Immunohistochemistry using Rockland's anti-TRPC6 monoclonal antibody shows detection of TRPC6 in human adrenal (cortex) tissue (40X). The antibody was used a dilution to 2.5 µg/mL. The image shows strong staining with minimal background staining. Tissue was formalin fixed and paraffin 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.



ELISA
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 (p/n MB-076), Rabbit Anti-Mouse IgG HRP conjugated (p/n 610-403-C46) and TMB substrate (p/n TMBE-1000).



Western Blot
Western Blot of Mouse Anti-TRPC6 Antibody. Lane 1: Opal Prestained Molecular Weight Marker (p/n MB-210-0500). Lane 2: Mouse Pancreas Tissue Lysate (p/n W10-000-T023) [10µg]. Lane 3: MCF-7 Whole Cell Lysate (p/n W09-000-360) [10µg]. Lane 4: A431 Whole Cell Lysate (p/n W09-000-361) [10µg]. Lane 5: Jurkat Whole Cell Lysate (p/n W09-001-370) [10µg]. Primary Antibody: Anti-TRPC6 at 1µg/mL overnight at 2-8°C. Secondary Antibody: Rabbit Anti-Mouse IgG Peroxidase (p/n 610-403-C46) 1:40000 for 30mins at RT. Blocking Buffer: BlockOut Buffer (p/n MB-073) for 30mins at RT. Predicted MW: ~30kDa. Observed MW: ~40kDa. Exposure: 5sec.



Western Blot
Western Blot of Mouse anti-TRPC6 Antibody. Lane 1: Mouse Kidney WCL (p/n W10-000-T017). Load: 10 µg per lane. Primary antibody: TRPC6 Antibody at 1:1000 for overnight at 4°C. Secondary antibody: donkey anti-mouse DyLight™ 649 (p/n 610-743-002) at 1:20,000 for 30 min at RT. Block: MB-070 for 30 min at RT.

References

- Alonso-González et al. Human Digital Meissner Corpuscles Display Immunoreactivity for the Multifunctional Ion Channels Trpc6 and Trpv4. *Anatomical record* (2017)

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