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Anti-ROBO-1 Antibody

Catalog Number: A01530-1

About ROBO1

ROBO-1 (also called Roundabout homolog 1 precursor and Deleted in U twenty twenty (DUTT)) functions as a receptor for SLIT1 and SLIT2. The SLIT proteins are thought to act as a molecular guidance cue in cellular migration, including axonal navigation at the ventral midline of the neural tube and projection of axons to different regions during neuronal development. In axon growth cones, the silencing of the attractive effect of NTN1 by SLIT2 may require the formation of a ROBO1-DCC complex. ROBO-1 may also be required for lung development. ROBO-1 is a type I membrane protein. ROBO-1 is a widely expressed protein with the exception of the kidney. Defects in ROBO1 may be a cause of breast and lung cancer. ROBO-1 maps within a region of overlapping homozygous deletions characterized in both small cell lung cancer cell lines (SCLC) and in a breast cancer cell line. Multiple splice variants have been identified for this protein.

Overview

Product Name	Anti-ROBO-1 Antibody
Reactive Species	Human, Mouse
Description	Boster Bio Anti-ROBO-1 Antibody (Catalog # A01530-1). Tested in ELISA, IHC, WB applications. This antibody reacts with Human, Mouse.
Application	ELISA, IP, IF, IHC, WB
Clonality	Polyclonal
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	Rabbit
Uniprot ID	Q2M1J3

Technical Details

Immunogen	This affinity-purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to an C-Terminal region near amino acids 1625-1650 of Human ROBO-1.
Predicted Reactive Species	Bovine, Mammalian
Isotype	IgG
Form	Liquid (sterile filtered)

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Concentration	0.98 mg/mL by UV absorbance at 280 nm
Purification	This affinity purified antibody is directed against human ROBO-1 protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest reactivity with this protein from human, mouse, rat and dog sources based on 100% homology for the immunogen sequence. Cross-reactivity will occur with all isoforms of ROBO-1. Cross-reactivity with ROBO-1 homologues from other sources has not been determined.
Suggested Dilutions	ELISA: 1:30,000 - 1:60,000 IHC: 2 µg/ml to 10 µg/ml IF Microscopy: User optimized IP: User optimized WB: 1:500 - 1:3,000 This affinity purified antibody has been tested for use in ELISA, western blot, and immunohistochemistry. It may be suitable for immunofluorescence and IP. Specific conditions for reactivity should be optimized by the end user. Expect a band at ~181 kDa in size corresponding to ROBO-1 by western blotting in the appropriate cell lysate or extract.

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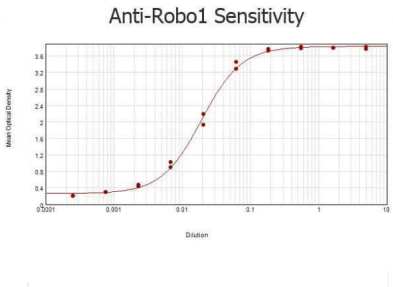
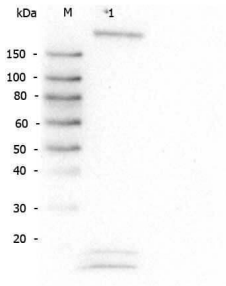
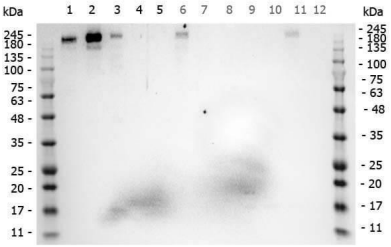
Anti-ROBO-1 Antibody (A01530-1) Images



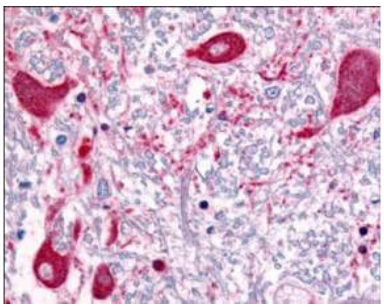
ELISA results of purified Rabbit anti-Robo-1 Antibody tested against BSA-conjugated peptide of immunizing peptide. Each well was coated in duplicate with 0.1µg of conjugate. The starting dilution of antibody was 5ug/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using 3% fish gel, Goat anti-Rabbit IgG Antibody Peroxidase Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) and TMB ELISA Peroxidase Substrate .



Western Blot of Rabbit anti-Robo-1 antibody. Lane M: Super Signal Molecular Weight Marker. Lane 1: HeLa WCL . Load: 30 µg lysate. Primary antibody: Robo-1 antibody at 1:1,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody at 1:40,000 for 30 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting for 30 min at RT. Predicted/Observed size: 181 kDa, 181 kDa for Robo-1. Other band(s): lower bands not identified.



Western Blot of Rabbit anti-ROBO1 antibody. Marker: Opal Pre-stained ladder . Lane 1: HEK293 lysate . Lane 2: HeLa Lysate . Lane 3: MCF-7 Lysate . Lane 4: Jurkat Lysate . Lane 5: A431 Lysate . Lane 6: A549 Lysate . Lane 7: LNCap Lysate . Lane 8: MOLT-4 Lysate . Lane 9: Ramos Lysate . Lane 10: Raji Lysate . Lane 11: A-172 Lysate . Lane 12: NIH/3T3 Lysate . Load: 35 µg per lane. Primary antibody: ROBO1 antibody at 1ug/mL overnight at 4C. Secondary antibody: Peroxidase rabbit secondary antibody at 1:30,000 for 60 min at RT. Blocking Buffer: 1% Casein-TTBS for 30 min at RT. Predicted/Observed size: 181kDa for ROBO1.



Boster's Affinity Purified anti-ROBO1 antibody was used at a concentration of 5 µg/ml to detect ROBO1 in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows staining of human brain tissue. Tissue was formalin-fixed and paraffin embedded. Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.

Western blot using Boster's Affinity Purified anti-ROBO-1 antibody shows detection of a band at ~181 kDa corresponding to ROBO-1 present in mouse brain lysate (arrowhead). Approximately 35 µg of lysate was separated by 4-8% SDS-PAGE and transferred onto nitrocellulose. After blocking the membrane was probed with the primary antibody diluted to 1:1,000. Reaction occurred 2h at room

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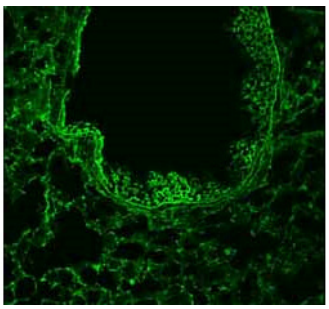
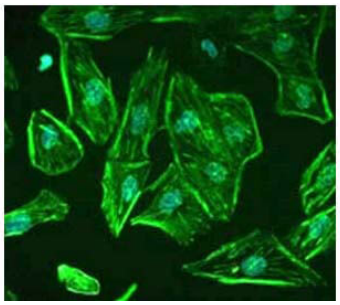
temperature followed by washes and reaction with a 1:10,000 dilution of IRDye™800 conjugated Gt-a-Rabbit IgG [H&L] MX for 45 min at room temperature. IRDye™800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.



1/50 staining mouse lung tissue sections (adult, frozen 100µm wholemount sections) by IHC-Fr. The tissue was paraformaldehyde fixed and permeabilized with triton x-100 before incubation with the antibody for 16 hours at 4°C.



Staining of ROBO1 in undifferentiated, immortalized human podocytes by Immunocytochemistry/ Immunofluorescence. Cells were fixed with 2% paraformaldehyde and 4% sucrose at room temperature for 10 minutes. The cells were then washed once with PBS, permeabilized with 0.3% Triton X-100 for 10 minutes and incubated with blocking solution (2% FCS, 2% BSA, 0.2% fish gelatin) for 30 minutes, before further incubation with primary Ab for 1 hour. An Alexa Fluor 488 goat anti-rabbit IgG secondary antibody was used at a dilution of 1/200. DAPI was used for nuclear counterstaining. Image from Lindenmeyer MT et al. Systematic Analysis of a Novel Human Renal Glomerulus-Enriched Gene Expression Dataset. PLoS One. 2010 July 12;5(7):e11545, Fig 5.

Anti-ROBO-1 Antibody

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