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Datasheet for 600-401-692

ROBO-1 Antibody

Overview

Description:	Anti-ROBO-1 (RABBIT) Antibody - 600-401-692
Item No.:	600-401-692
Size:	100 µg
Applications:	ELISA, IHC, WB, IF
Reactivity:	Human, Mouse
Host Species:	Rabbit

Product Details

Background:	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.
Synonyms:	rabbit anti-ROBO1 antibody, ROBO 1, ROBO-1, hROBO-1, Roundabout homolog 1, Deleted in U twenty twenty, DUTT1, DUTT-1
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	ROBO1
Reactivity:	Human, Mouse

Immunogen Type:	Conjugated Peptide
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.
Purity/Specificity:	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.
Relevant Links:	• UniProtKB - Q2M1J3 • NCBI - AAI12337.1 • GeneID - 6091

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IF (Based on references)
Application Note:	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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:30,000 - 1:60,000
IF:	User Optimized
IHC:	2 µg/ml to 10 µg/ml
IP:	User Optimized
WB:	1:500 - 1:3,000

Formulation

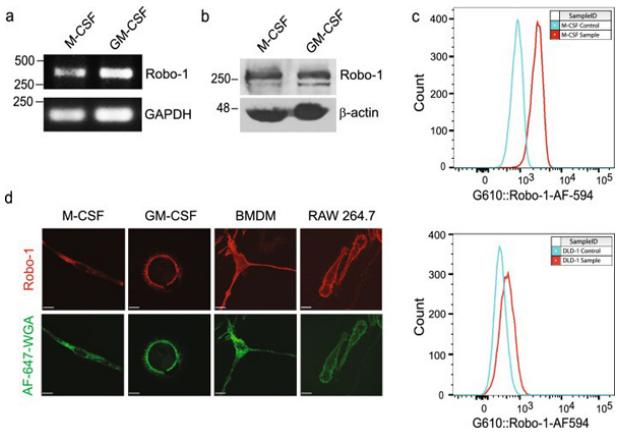
Physical State:	Liquid (sterile filtered)
Concentration:	1.03 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

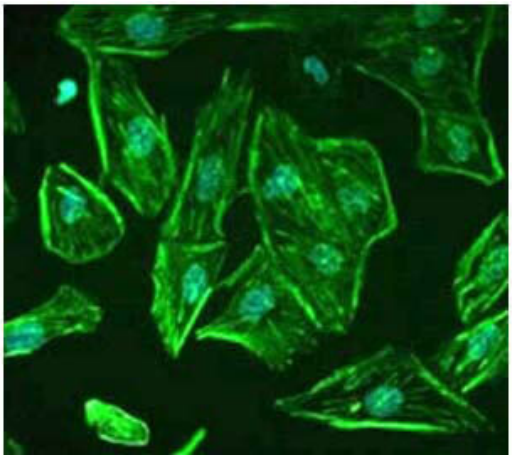
Shipping & Handling

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.

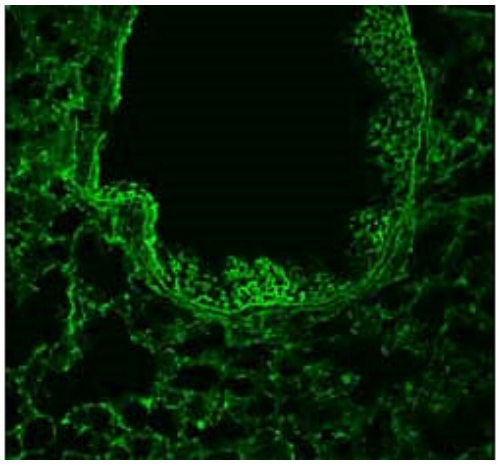
Images



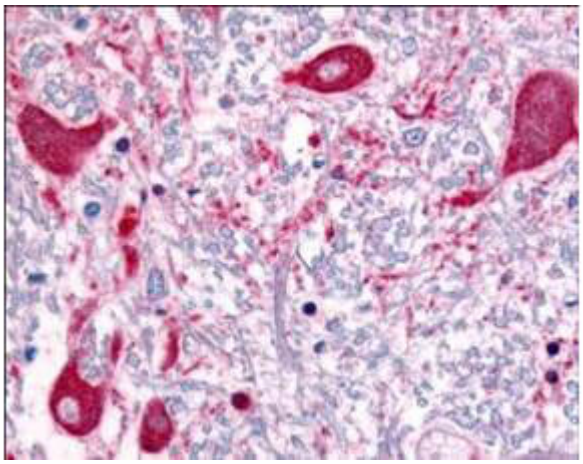
Immunofluorescence Microscopy
Human and murine macrophages express the Slit2 receptor, Robo-1. (a), Human peripheral blood mononuclear cells (PBMC) were isolated and incubated with M-CSF or GM-CSF to generate M-CSF- or GM-CSF-induced macrophages, respectively. RT-PCR was performed using specific primers for Robo-1 and GAPDH. (b), Lysates were collected from M-CSF- and GM-CSF-induced human macrophages and immunoblotting was performed using antibodies specifically detecting Robo-1 or ß-actin. (c), M-CSF-induced human macrophages (top) and DLD-1 cells (bottom) were first incubated with viability dye followed by AF-594-conjugated secondary Ab (Control, blue peak), or anti-Robo-1 Ab (p/n 600-401-692) and AF-594-conjugated secondary Ab (Sample, red peak). Data from live, single cells was acquired using an LSRII flow cytometer (BD Biosciences-US) with FACSDiva software (BD Biosciences-US) and analyzed with FlowJo v10 (BD Biosciences-US). (d), M-CSF- and GM-CSF-induced human macrophages, murine BMDM and RAW264.7 macrophages were fixed and incubated with anti-Robo-1 Ab (p/n 600-401-692), followed by Dylight549-conjugated secondary Ab (p/n 610-742-002) (red) and AF-647-conjugated wheat germ agglutinin (pseudocoloured green). Cells were imaged using a spinning disk confocal microscope at 63× magnification. Scale bar, 10 µm. Fig 1.
PMID: 33574432



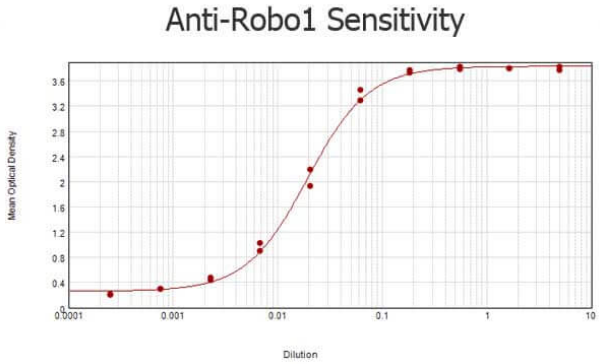
Immunofluorescence Microscopy
Staining of ROBO1 in undifferentiated, immortalized human podocytes by Immunocytochemistry/ Immunofluorescence. Cells were fixed with 2% paraformaldehyde and 4% sucrose at room temperature for 10 minutes. The cells were then washed once with PBS, permeabilized with 0.3% 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.



Immunofluorescence Microscopy
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.

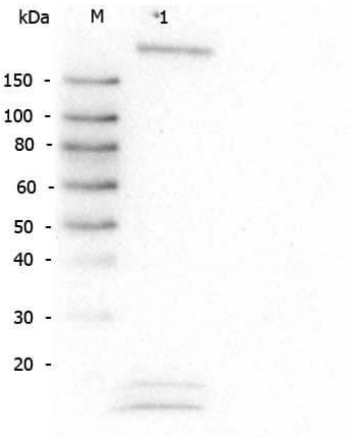


Immunohistochemistry
Rockland's Affinity Purified anti-ROBO1 antibody was used at a concentration of 5 µg/ml to detect ROBO1 in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows staining of human brain tissue. Tissue was formalin-fixed and paraffin embedded.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

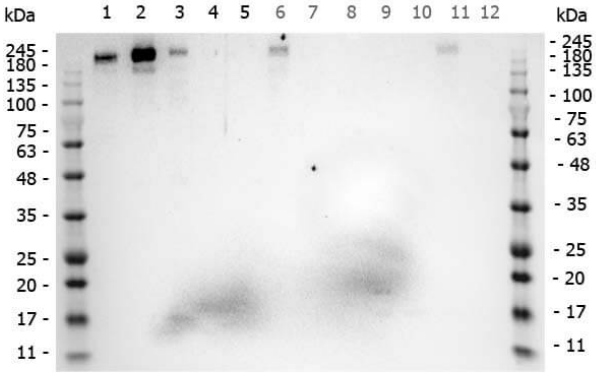


ELISA
ELISA results of purified Rabbit anti-Robo-1 Antibody tested against BSA-conjugated peptide of immunizing peptide. Each well was coated in duplicate with 0.1µg of conjugate. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using 3% fish gel, Goat anti-Rabbit IgG Antibody Peroxidase Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) (p/n 611-103-122) and TMB ELISA Peroxidase Substrate (p/n TMBE-1000).

Western Blot
Western blot using Rockland's Affinity Purified anti-ROBO-1 antibody shows detection of a band at ~181 kDa corresponding to ROBO-1 present in mouse brain lysate (p/n W10-000-T004) (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 temperature followed by washes and reaction with a 1:10,000 dilution of IRDye™800 conjugated Gt-a-Rabbit IgG [H&L] MX (p/n 611-132-122) 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.



Western Blot
Western Blot of Rabbit anti-Robo-1 antibody. Lane M: Super Signal Molecular Weight Marker. Lane 1: HeLa WCL (p/n W09-000-364). Load: 30 µg lysate. Primary antibody: Robo-1 antibody at 1:1,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:40,000 for 30 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting (p/n MB-070) for 30 min at RT. Predicted/Observed size: 181 kDa, 181 kDa for Robo-1. Other band(s): lower bands not identified.



Western Blot

Western Blot of Rabbit anti-ROBO1 antibody. Marker: Opal Pre-stained ladder (p/n MB-210-0500). Lane 1: HEK293 lysate (p/n W09-000-365). Lane 2: HeLa Lysate (p/n W09-000-364). Lane 3: MCF-7 Lysate (p/n W09-000-360). Lane 4: Jurkat Lysate (p/n W09-000-370). Lane 5: A431 Lysate (p/n W09-000-361). Lane 6: A549 Lysate (p/n W09-001-372). Lane 7: LNCap Lysate (p/n W09-001-GJ9). Lane 8: MOLT-4 Lysate (p/n W09-001-GK2). Lane 9: Ramos Lysate (p/n W09-000-GK4). Lane 10: Raji Lysate (p/n W09-001-368). Lane 11: A-172 Lysate (p/n W09-001-GL5). Lane 12: NIH/3T3 Lysate (p/n W10-000-358). Load: 35 µg per lane. Primary antibody: ROBO1 antibody at 1ug/mL overnight at 4C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:30,000 for 60 min at RT. Blocking Buffer: 1% Casein-TTBS (p/n MB-082) for 30 min at RT.
Predicted/Observed size: 181kDa for ROBO1.

References

- Yusuf B et al. The neurorepellent, Slit2, prevents macrophage lipid loading by inhibiting CD36-dependent binding and internalization of oxidized low-density lipoprotein. *Sci Rep.* (2021)
- Pluthero FG et al. Imaging Platelets and Megakaryocytes by High-Resolution Laser Fluorescence Microscopy. *Methods Mol Biol.* (2018)

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