

Certificate of Analysis

Product: Affinity Purified Anti-ROBO-1 [Rabbit]

Code: 600-401-692

Lot #: 20000

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

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.

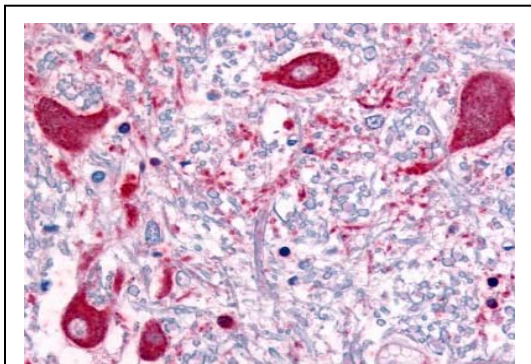


Figure 2. Immunohistochemistry. Rockland's Affinity Purified anti-ROBO1 antibody was used at a concentration of 5 µg/ml to detect ROBO1 in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows staining of human brain tissue. Tissue was formalin-fixed and paraffin embedded. *Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.*

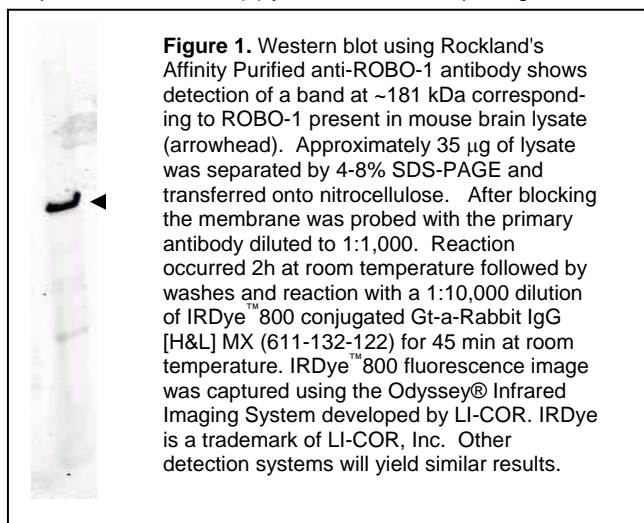


Figure 1. Western blot using Rockland's Affinity Purified anti-ROBO-1 antibody shows detection of a band at ~181 kDa corresponding to ROBO-1 present in mouse brain lysate (arrowhead). Approximately 35 µg of lysate was separated by 4-8% SDS-PAGE and transferred onto nitrocellulose. After blocking the 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 (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.

Application Note(s): This affinity purified antibody has been tested for use in ELISA, immunohistochemistry and western blot. 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.

Recommended Dilutions:

ELISA	1:30,000 - 1:160,000
WESTERN BLOT	1:500 - 1:3,000
IMMUNOHISTOCHEMISTRY	2 µg/ml to 10 µg/ml
OTHER APPLICATIONS	User Optimized

Purity and 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 Link(s): Swis-Prot [Q9Y6N7](#)

NCBI Link [NP_002932](#) (isoform a)

Protein Sequence: Human ROBO-1, 1651 aa, predicted MW 180.9 kDa

1	mkwkhvplv	misllslspn	hlflaqlipd	pedvergnhd	gtpiptsdnd	dnslygtgr
61	lrqedfpri	vehpsdlivs	kgepatlnck	aegrptpie	wykggervet	dkddprshrm
121	llpsgsiffl	rivhgrksrp	degvyvcvar	nylgeavshn	aslevaird	dfrqnpdsvm
181	vavgepavme	cqpprghpep	tiswkkdgs	lddkderiti	rggklmityt	rksdagkyvc
241	vgtnmvgere	sevaeltvle	rpsfvkrpsn	lavtvdsae	fkceargdpv	ptvrwrkddg
301	elpksryeir	ddhtlkirkv	tagdmgsytc	vaenmvgkae	asatltvqep	phfvvkprdq
361	vvalgrtvtf	qceatgnppq	aifwrregsq	nllfsyqqpq	sssrfsvsqt	gdltitnvqr
421	sdvgyyicqt	lnvagsiitk	aylevtdvia	drpppvirag	pvnqtavavd	tfvlscvatg

1081	snlsnmmng	sgdsgekhwk	plgqqkqeva	pvqyniveqn	klmkdyrand	tvptipynq
1141	sydqntggsy	nssdrsgsts	gsqghkkgar	tpkvpkqggm	nwadllpppp	ahppphsnse
1201	eynisvdesy	dqempcvppp	armylqqdel	eeeeergpt	ppvrngaassp	aavsyshqst
1261	atltspqee	lqpmldqcp	etghmqhqp	rrrpvsppp	pprpispht	ygysgplvs
1321	dmdtdapeee	edeadmekak	mqtrlllrg	leqtpassvg	dlessvtgsm	ingwgsasee
1381	dnissgrsv	sssdgsfftd	adfaqavaaa	aeyaglkvar	rqmqdaagr	hfhasqcprp
1441	tspvdsnm	saavmqktrp	akklkhqpg	lrretyddl	ppppvpppai	ksptaqsqtq
1501	levrvvvpk	lpsmdartdr	ssdrkgssyk	grevldgrqv	vdmrntpgdp	reaqqndg
1561	kgrgnkaakr	dlppakthli	qedilpypcrp	tfptsnnprd	psssssmssr	gsgsrqreqa
1621	nvgrniaem	qvlgygerge	dnneelete	s		

Immunogen: This affinity-purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 1632-1644 of Human ROBO-1.

General References:

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- Hivert,B., Liu,Z., Chuang,C.Y., Doherty,P. and Sundaresan,V. (2002) Robo1 and Robo2 are homophilic binding molecules that promote axonal growth. *Mol. Cell. Neurosci.* **21** (4), 534-545.
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- Yuan,W., Zhou,L., Chen,J.H., Wu,J.Y., Rao,Y. and Ornitz,D.M. (1999) The mouse SLIT family: secreted ligands for ROBO expressed in patterns that suggest a role in morphogenesis and axon guidance. *Dev. Biol.* **212** (2), 290-306.
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- #[MB-070](#) Blocking Buffer for Fluorescent Western Blotting
- # [KIA-003](#) **MaxTag**TM Anti-RABBIT IgG Kit for Immunoblotting

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