

# Product Datasheet

## ROBO1 Antibody - BSA Free

### NB600-1254

Unit Size: 0.1 mg

Store at -20C short term. Aliquot and store at -80C long term. Avoid freeze-thaw cycles.

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**NB600-1254**

ROBO1 Antibody - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Store at -20C short term. Aliquot and store at -80C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.01% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Product Description	
Description	<p>This affinity purified antibody is directed against human ROBO1 protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification</p> <p>Store vial at -20C prior to opening. Aliquot contents and freeze at -20C 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 4C as an undiluted liquid. Dilute only prior to immediate use.</p>
Host	Rabbit
Gene ID	6091
Gene Symbol	ROBO1
Species	Human, Mouse
Reactivity Notes	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 ROBO1. Cross reactivity with ROBO1 homologues from other sources has not been determined.
Specificity/Sensitivity	This affinity purified antibody is directed against human ROBO1 protein. 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 ROBO1. Cross reactivity with ROBO1 homologues from other sources has not been determined.
Immunogen	This affinity-purified ROBO1 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 ROBO1. (Uniprot: Q2M1J3)

Product Application Details	
Applications	Western Blot, ELISA, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500-1:3000, ELISA 1:30000-1:60000, Immunohistochemistry 2 ug/ml-10 ug/ml, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunoprecipitation 1:10 - 1:500, Immunohistochemistry-Paraffin 1:10-1:500

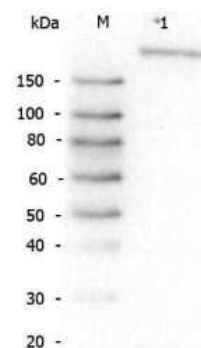


**Application Notes**

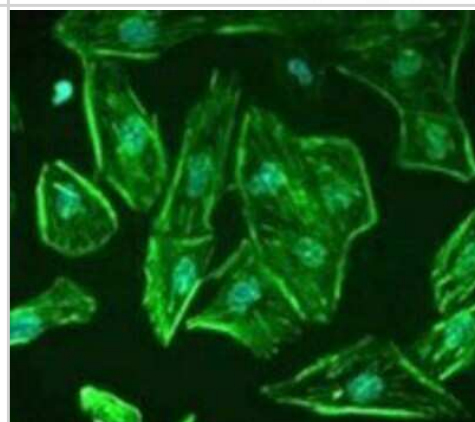
This affinity purified antibody has been tested for use in ELISA, western blot, and immunohistochemistry. It may be suitable for immunofluorescence and IP. Specific conditions for reactivity should be optimized by the end user. Expect a band at ~181 kDa in size corresponding to ROBO-1 by western blotting in the appropriate cell lysate or extract.

**Images**

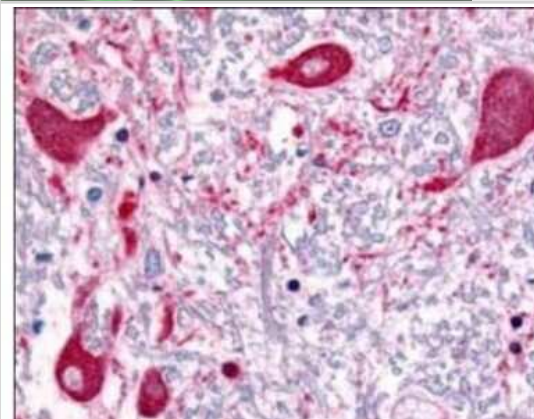
**Western Blot: ROBO1 Antibody [NB600-1254]** - Western Blot of ROBO1 antibody. Lane M: Super Signal Molecular Weight Marker. Lane 1: HeLa WCL. Load: 30 ug lysate. Primary antibody: ROBO1 antibody at 1:1,000 for overnight at 4C. Secondary antibody: Peroxidase rabbit secondary antibody at 1:40,000 for 30 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting for 30 min at RT. Predicted/Observed size: 181 kDa, 181 kDa for ROBO1. Other band(s): lower bands not identified.



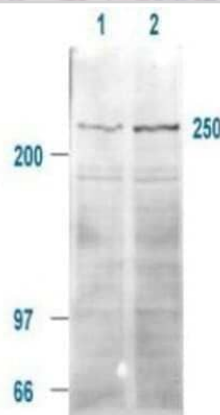
**Immunocytochemistry/Immunofluorescence: ROBO1 Antibody [NB600-1254]** - Staining of Robo1 in undifferentiated, immortalized human podocytes by Immunocytochemistry/ Immunofluorescence. Cells were fixed with 2% paraformaldehyde and 4% sucrose at room temperature for 10 minutes. The cells were then washed 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.



**Immunohistochemistry: ROBO1 Antibody [NB600-1254]** - Affinity Purified ROBO1 antibody was used at a concentration of 5 ug/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.



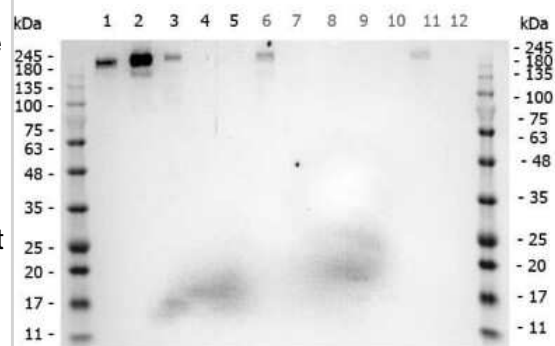
**Western Blot: ROBO1 Antibody [NB600-1254]** - Biotinylated human mTOR present in a 293T whole cell lysates. Cells were serum-starved for 24 hours prior to harvest. ~20ug of lysate was loaded per lane for SDS-PAGE. Untreated cells are shown in lane 1, whereas cells in lane 2 were treated with IGF-1 (100 ng/ml) for 20 min prior to harvest. Follow reaction of antibody with a 1:2000 dilution of HRP Goat-a-Rabbit IgG for visualization.



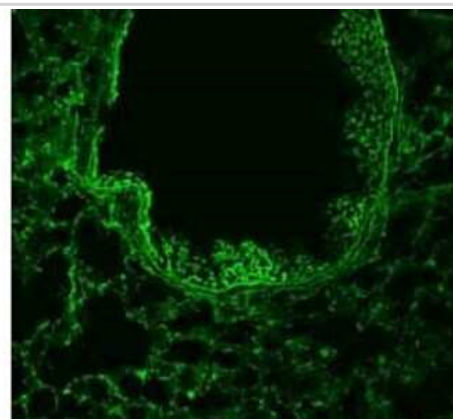
**Western Blot: ROBO1 Antibody [NB600-1254]** - Western blot using Affinity Purified ROBO1 antibody shows detection of a band at ~181 kDa corresponding to ROBO1 present in mouse brain lysate (arrowhead). Approximately 35 ug of lysate was separated by 4-8% SDS-PAGE and transferred onto nitrocellulose. 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(TM)800 conjugated Gt-a-Rabbit IgG [H&L] MX for 45 min at room temperature. IRDye(TM)800 fluorescence image was captured using the Odyssey(R) Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.



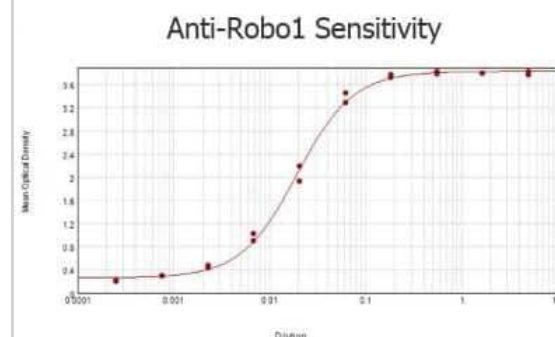
**Western Blot: ROBO1 Antibody [NB600-1254]** - Western Blot of ROBO1 antibody. Marker: Opal Pre-stained ladder. Lane 1: HEK293 lysate. Lane 2: HeLa Lysate. Lane 3: MCF-7 Lysate. Lane 4: Jurkat Lysate. Lane 5: A431 Lysate. Lane 6: A549 Lysate. Lane 7: LNCap Lysate. Lane 8: MOLT-4 Lysate. Lane 9: Ramos Lysate. Lane 10: Raji Lysate. Lane 11: A-172 Lysate. Lane 12: NIH/3T3 Lysate. Load: 35 ug 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.



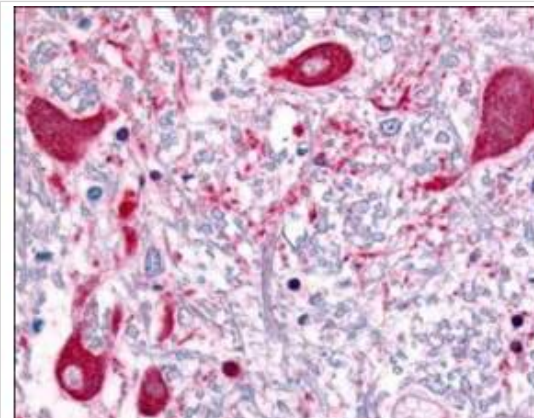
**Immunohistochemistry: ROBO1 Antibody [NB600-1254]** - 1/50 staining mouse lung tissue sections (adult, frozen 100um wholemount sections) by IHC-Fr. The tissue was paraformaldehyde fixed and permeabilized with triton x-100 before incubation with the antibody for 16 hours at 4C.



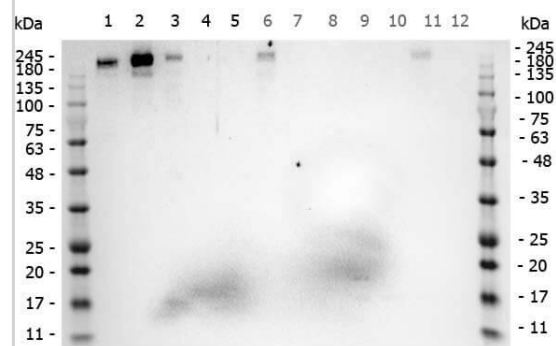
**ELISA: ROBO1 Antibody [NB600-1254]** - ELISA results of purified ROBO1 antibody tested against BSA-conjugated peptide of immunizing peptide. Each well was coated in duplicate with 0.1ug of conjugate. The starting dilution of antibody was 5ug/ml and the X-axis represents the Log10 of a 3-fold dilution. 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.



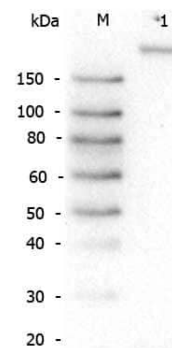
Affinity Purified anti-ROBO1 antibody was used at a concentration of 5 ug/ml to detect ROBO1 in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows staining of human brain tissue. Tissue was formalin-fixed and paraffin embedded. Personal Communication, Tina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



Western Blot of ROBO1 Antibody antibody. Marker: Opal Pre-stained ladder



Western Blot of Rabbit anti-Robo-1 antibody. Lane M: Super Signal Molecular Weight Marker. Lane 1: HeLa WCL







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### **Products Related to NB600-1254**

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HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP2-55652PEP	ROBO1 Recombinant Protein Antigen

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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