abcam

ab274385

Anti-Robo1 antibody [EPR23699-159] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR23699-159
Purification technique	Affinity purification Protein A
Concentration	1.008 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Not recommended

Species	Mouse, Rat, Human
Dilution info	-

Notes	-

IP

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

IHC-Fr

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Target data

See full target information ROBO1

Function	Receptor for SLIT1 and SLIT2 that mediates cellular responses to molecular guidance cues in cellular migration, including axonal navigation at the ventral midline of the neural tube and projection of axons to different regions during neuronal development (PubMed:10102268, PubMed:24560577). Interaction with the intracellular domain of FLRT3 mediates axon attraction towards cells expressing NTN1 (PubMed:24560577). In axon growth cones, the silencing of the attractive effect of NTN1 by SLIT2 may require the formation of a ROBO1-DCC complex (By similarity). Plays a role in the regulation of cell migration via its interaction with MYO9B; inhibits MYO9B-mediated stimulation of RHOA GTPase activity, and thereby leads to increased levels of active, GTP-bound RHOA (PubMed:26529257). May be required for lung development (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab274385 is the carrier-free version of ab256791.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The Roundabout homolog 1 (Robo1) also known as DUTT1 is a transmembrane receptor protein weighing approximately 181 kDa. It is part of the Robo receptor family specifically involved in axon guidance and neuronal migration. Robo1 primarily appears in neuronal tissues and other tissues like the lungs kidneys and prostate. Its role involves dynamic interactions with several ligands such as Slit proteins leading to the modulation of cellular pathways.
Biological function summary	Robo1 interacts with Slit proteins to control processes like repulsion of axonal growth cones during neuronal development. The protein acts as an important player in guiding neuronal connections and establishing boundaries. Robo1 does not function alone forming complexes with other receptors and cell adhesion molecules to execute its biological roles effectively. Structurally this receptor facilitates cellular responses to environmental cues through its cytoplasmic signaling cascade.
Pathways	Robo1 engages in the Slit-Robo signaling pathway essential for axonal guidance and cell migration. This pathway involves several key interactions with proteins

	such as DCC (Deleted in Colorectal Carcinoma) and Netrin impacting neuronal positioning and differentiation. Additionally Robo1 influences cellular orientation and tissue architecture by participating in the actin cytoskeleton remodeling pathway further demonstrating its significance in neuronal patterning.
Associated diseases and disorders	Robo1 has links to cancer and neurodevelopmental disorders like autism spectrum disorders. The dysregulation of Robo1 expression has associations with aggressive tumor growth particularly in breast and lung cancers where altered signaling influences metastatic potential. Furthermore Robo1 interacts with proteins like Slit2 in cancer progression highlighting its importance in these conditions. In neurodevelopmental disorders altered Robo1 signaling can affect neural circuit formation contributing to changes seen in conditions like autism.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

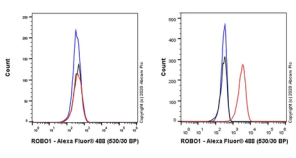
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

7 product images



Flow Cytometry - Anti-Robo1 antibody [EPR23699-159] - BSA and Azide free (ab274385)

Robo1 Flow Cytometry staining using rabbit Anti-Robo1 antibody

This data was developed using ab256791, the same antibody clone in a different buffer formulation.
Flow cytometric analysis of LoVo (Human colorectal adenocarcinoma epithelial cell) (Left) / HeLa (Human cervix adenocarcinoma epithelial cell) (Right) cells labelling Robo1 with ab256791 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.

Negative control: LoVo(PMID: 16740745).

Gated on viable cells.



Immunoprecipitation - Anti-Robo1 antibody [EPR23699-159] - BSA and Azide free (ab274385)

This data was developed using ab256791, the same antibody clone in a different buffer formulation.
Robo1 was immunoprecipitated from 0.35 mg Mouse E14.5 brain tissue lysate with ab256791 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab256791 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/1000 dilution.

Lane 1: Mouse E14.5 brain tissue lysate 10 ug.

Lane 2: ab256791 IP in Mouse E14.5 brain tissue lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab256791 in mouse E14.5 brain tissue lysate.

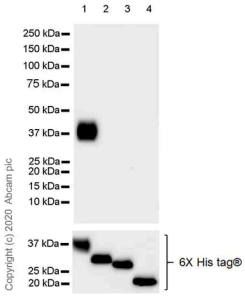
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

All lanes:
Immunoprecipitation - Anti-Robo1 antibody [EPR23699-159] (ab256791)

Predicted band size: 181 kDa

Observed band size: 120 kDa



Western blot - Anti-Robo1 antibody [EPR23699-159] - BSA and Azide free (ab274385)

Robo1 Western blot staining using rabbit Anti-Robo1 antibody

This data was developed using ab256791, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

These recombinant proteins were made in house. The sample loaded onto lane 1 was purified Robo1 recombinant protein expressed from a mammalian - HEK-293 expression system. The samples loaded onto lanes 2-4 were E.coil extracts containing Robo2, Robo3 or Robo4 recombinant protein respectively.

Exposure time: 26 seconds.

All lanes:
Western blot - Anti-Robo1 antibody [EPR23699-159] (ab256791) at 1/1000 dilution

Lane 1:
His-tagged human Robo1 recommbinant protein (aa660-897(Q883R)) at 20 µg

Lane 2:
His-tagged human Robo2 recommbinant protein (aa621-859) at 10 µg

Lane 3:
His-tagged human Robo3 recommbinant protein (aa655-892) at 50 µg

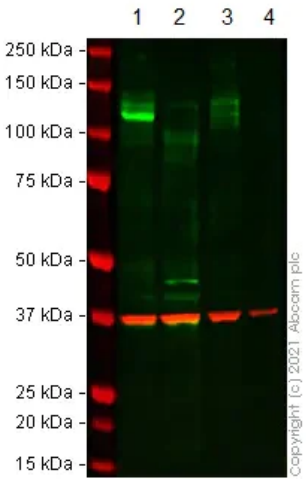
Lane 4:
His-tagged human Robo4 recommbinant protein (aa301-463) at 30 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000 dilution

Predicted band size: 181 kDa

Observed band size: 38 kDa



Western blot - Anti-Robo1 antibody [EPR23699-159] - BSA and Azide free (ab274385)

This data was developed using the same antibody clone in a different buffer formulation (ab256791).
Lanes 1 - 4: Merged signal (red and green). Green - ab256791 observed at 120 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab256791 was shown to react with Robo1 in western blot. The band observed in the CRISPR/Cas9 edited lysate lane below 120 kDa is likely to represent a truncated form. This has not been investigated further. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab256791 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

All lanes:
Western blot - Anti-Robo1 antibody [EPR23699-159] (ab256791) at 1/1000 dilution

Lane 1:
Wild-type HAP1 cell lysate at 20 µg

Lane 2:
ROBO1 CRISPR/Cas9 edited HAP1 cell lysate at 20 µg

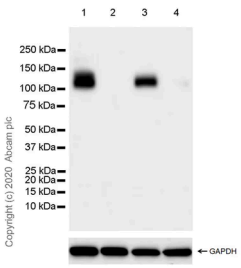
Lane 3:
HeLa cell lysate at 20 µg

Lane 4:
MCF7 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 181 kDa

Observed band size: 120 kDa



Western blot - Anti-Robo1 antibody [EPR23699-159] - BSA and Azide free (ab274385)

This data was developed using ab256791, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID: 20471383, 16740745). The 120/130-kDa bands correspond to the Robo1 cleavage fragments from metalloprotease and/or gamma secretase activities (PMID: 20570941, 20471383).

Negative control: LoVo (PMID: 16740745); **Low expression:** MCF7 (PMID: 16740745).

Lysates used in this blot have experienced a freeze-thaw cycle.

Exposure time: 10 seconds.

All lanes:
Western blot - Anti-Robo1 antibody [EPR23699-159] (ab256791) at 1/1000 dilution

Lane 1:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
LoVo (human colorectal adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

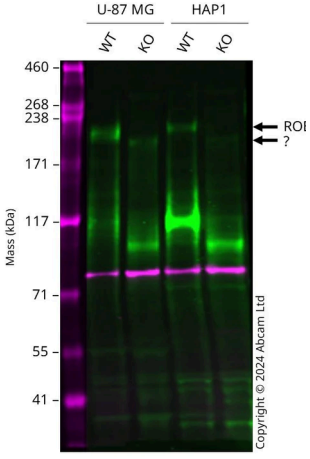
Lane 4:
MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/50000 dilution

Predicted band size: 181 kDa

Observed band size: 120-130 kDa



Western blot - Anti-Robo1 antibody [EPR23699-159] - BSA and Azide free (ab274385)

This data was developed using the same antibody clone in a different buffer formulation (ab256791).

Western blot: Anti-ROBO1 antibody [EPR23699-159] (ab256791) staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab256791 was shown to bind specifically to ROBO1. A band was observed at 215 kDa in wild-type U-87 MG cell lysates with no signal observed at this size in ROBO1 knockout cell line. The identity of the lower band is unknown. To generate this image, wild-type and ROBO1 knockout U-87 MG cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room

temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-Robo1 antibody [EPR23699-159] (ab256791) at 1/1000 dilution

Lane 1:
Wild-type U-87 MG cell lysate at 20 µg

Lane 2:
ROBO1 knockout U-87 MG cell lysate at 20 µg

Lane 3:
Wild-type HAP1 cell lysate at 20 µg

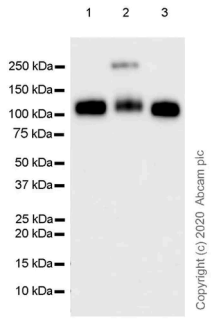
Lane 4:
ROBO1 knockout HAP1 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 215 kDa



Western blot - Anti-Robo1 antibody [EPR23699-159] - BSA and Azide free (ab274385)

Robo1 Western blot staining using rabbit Anti-Robo1 antibody

This data was developed using ab256791, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The 120 kDa band corresponds to the Robo1 cleavage fragments from metalloprotease and/or gamma secretase activities (PMID: 20570941, 20471383).

Lysates used in this blot have experienced a freeze-thaw cycle.

Exposure time: 70 seconds.

All lanes:
Western blot - Anti-Robo1 antibody [EPR23699-159] (ab256791) at 1/1000 dilution

Lane 1:
Mouse E14.5 brain tissue lysate at 20 µg

Lane 2:
C57 E18 brain tissue lysate at 20 µg

Lane 3:
Rat E14 brain tissue lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/50000 dilution

Predicted band size: 181 kDa

Observed band size: 120 kDa, 250 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.