Product Datasheet

Anti-ROBO3 [IPI-ROBO3.124]

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Overview

Antigen	ROBO3
Immunogen	Purified recombinant fragment of Human ROBO3, corresponding to AA: 21-891.
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-ROBO3.124
RRID	AB_3720935
IPI ID	TAB0017622-013-002
Specificity	ROBO3; Does not recognize other ROBOs
Species reactivity	human and mouse
Amount	100 µg
Concentration	1 mg/mL
Purification	Expressed in HEK293T cells and affinity purified using Protein A
Storage buffer	PBS, pH 7.4
Shipping	Shipped on blue ice at +4C
Storage	Store at +4C for up to 3 months. For long-term storage, aliquot and store at -20C. Avoid multiple freeze/thaw cycles.

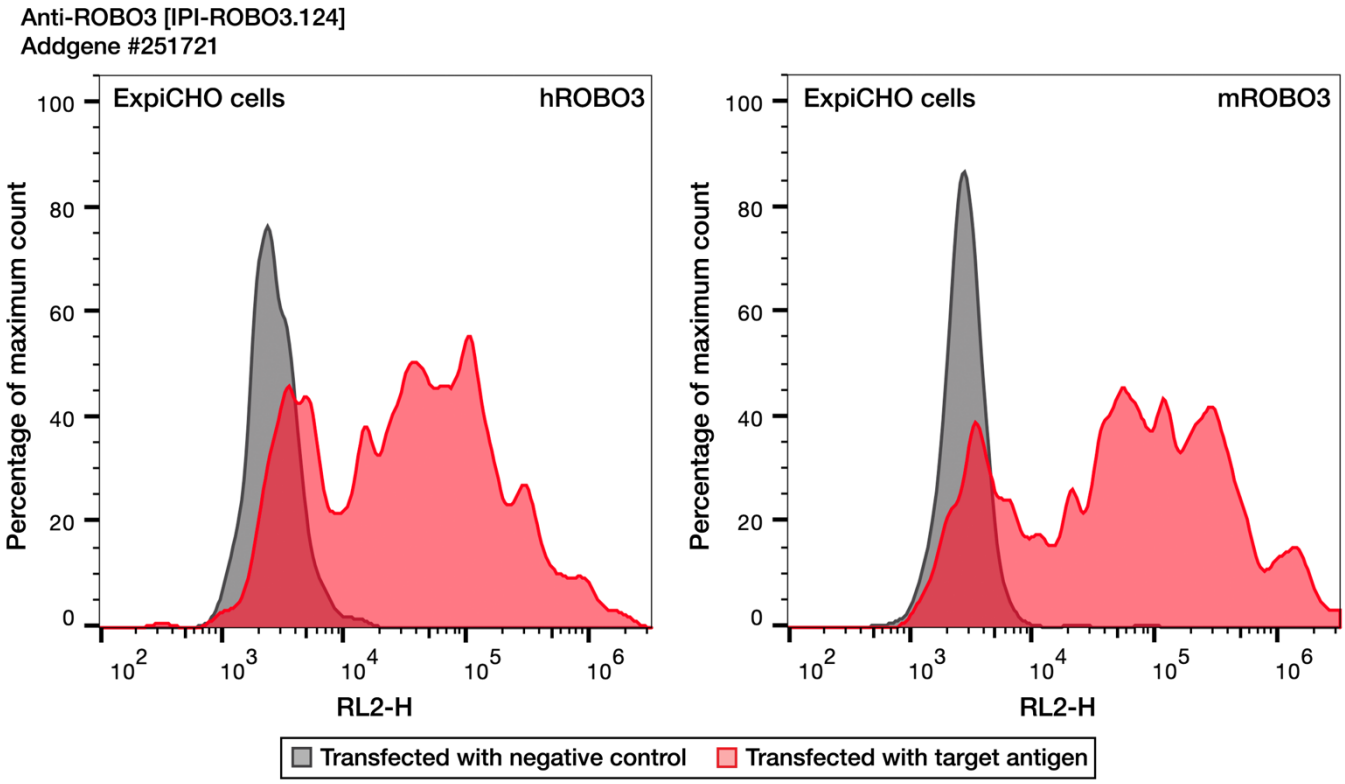
IPI Tested Applications‡

Application	Tested concentration	Result	Reference
Flow	0.66-100 µg/mL	Positive	https://doi.org/10.57733/addgene.vpcdbk
SPR	1 µg/mL	Positive	https://doi.org/10.57733/addgene.uupfyd
IF – Binding	1 µg/mL	Positive	https://doi.org/10.57733/addgene.k6fbks
IF – Specificity	1 µg/mL	Positive	https://doi.org/10.57733/addgene.d4lio3
IHC	10 µg/mL	Positive	https://doi.org/10.57733/addgene.7js33w

‡ Not suitable for WB application.

Applications

Flow cytometry

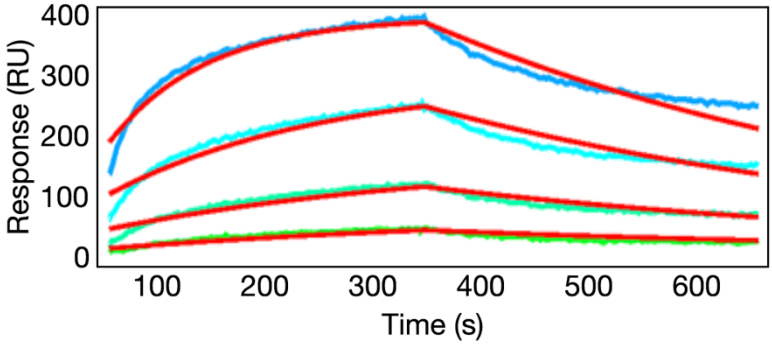


Anti-ROBO3 [IPI-ROBO3.124] (Addgene #251721) recognizes human and mouse ROBO3. Histogram from FACS analysis on ExpiCHO cells transfected with human or mouse ROBO3 (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) ROBO3 were labeled with Anti-ROBO3 [IPI-ROBO3.124] and Alexa Fluor 647 F(ab')2 goat anti-rabbit IgG Fc fragment (Jackson ImmunoResearch, 111-606-046). Labeled cells were analyzed with an Intellicyt iQue Screener Plus flow cytometer. Histograms were generated and normalized to mode using FlowJo™ v10.10. doi: https://doi.org/10.57733/addgene.vpcdbk

EC_{50} (data not shown): A fourteen-point titration of antibody concentrations, ranging from 660 nM (0.1 mg/mL) to 4.42 pM with a 1:2.5 dilution factor, against human and mouse ROBO3 showed reactivity towards human and mouse ROBO3 with observed EC50 values of 0.4 nM (Human), 0.47 nM (Mouse).

Surface Plasmon Resonance (SPR)

Anti-ROBO3 [IPI-ROBO3.124]
Addgene #251721

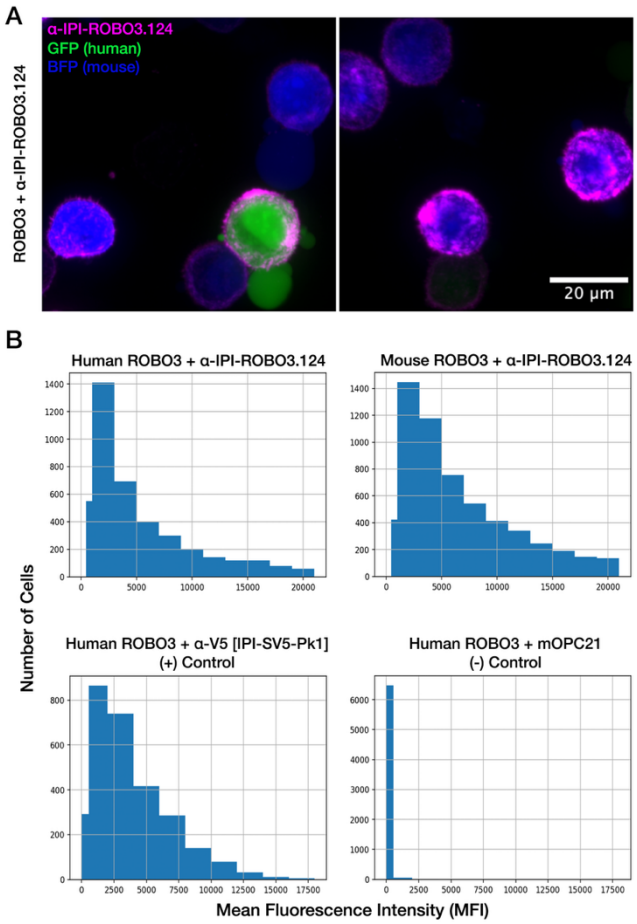


Kinetic Parameters

k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
$6.9x10^4$	$2.0x10^{-3}$	$3.0x10^{-8}$

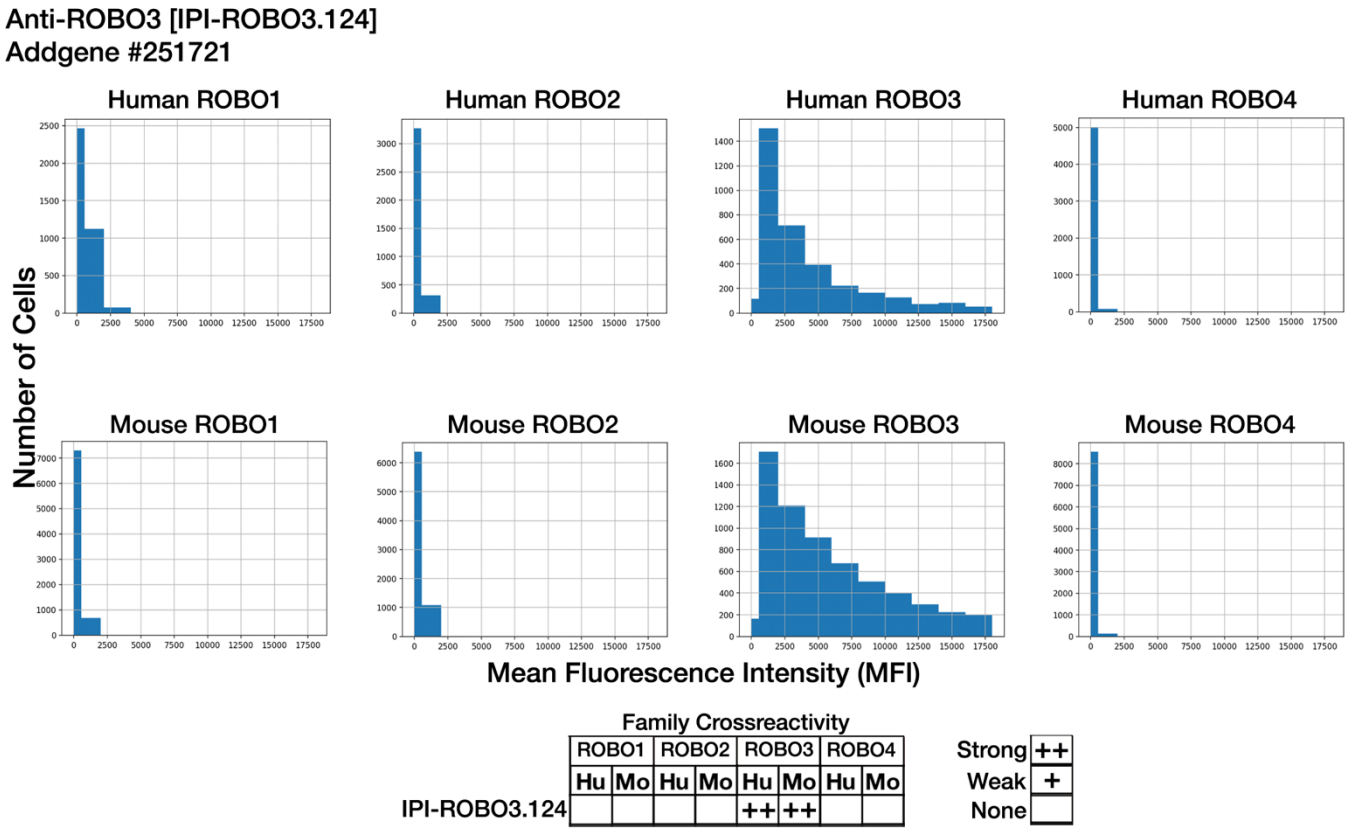
Surface Plasmon Resonance (SPR) kinetics analysis of the interaction between Anti-ROBO3 [IPI-ROBO3.124] and Human ROBO3. SPR binding kinetics were measured on a Carterra LSA using HC30M chips (Carterra, cat. #4279) at 25 °C. Goat anti-rabbit IgG Fc (Jackson ImmunoResearch, cat. #111-005-046) was immobilized via amine coupling, and test antibodies were captured using a 96-channel print-head. Antigens (400 nM to five lower concentrations, 2-fold dilutions) were injected in antigen buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.005% Tween 80) with 300 s association/ dissociation phases and acid regeneration. Data (reference/buffer subtracted, smoothed) were globally fit to a 1:1 Langmuir model to derive ka, kd, and KD using Carterra Kinetics software v1.9.2.44.63, and replotted in OriginPro 2023b. Results show a high-affinity and specific binding event between the antibody and antigen. doi: https://doi.org/10.57733/addgene.uupfyd

Immunofluorescence (IF) – Species Reactivity



Anti-ROBO3 [IPI-ROBO3.124] (Addgene#251721) shows binding to human and mouse ROBO3. A) Immunofluorescence (IF) of ExpiCHO cells transfected with human (Green/GFP) and mouse (Blue/BFP) ROBO3 stained with IPI-ROBO3.124 (magenta). Confocal images taken at 40X magnification on the ImageXpress confocal HT.ai microscope. Scale bar = 20 uM. B) Combined quantification of multiple images of the same transfected cells taken at 10X magnification. GFP- or BFP-positive cells were identified via the neural network CellPose, then the mean fluorescence intensity (MFI) of the far-red channel for each cell, representing IPI-ROBO3.124 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 22000. IPI-ROBO3.124 staining of human and mouse ROBO3 is shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-ROBO3.124 was used at 1 ug/mL (1:1,000 dilution). doi: https://doi.org/10.57733/addgene.k6fbks

Immunofluorescence (IF) – Target Specificity



Family Crossreactivity

	ROBO1		ROBO2		ROBO3		ROBO4	
	Hu	Mo	Hu	Mo	Hu	Mo	Hu	Mo
IPI-ROBO3.124					++	++		

Strong	++
Weak	+
None	

Anti-ROBO3 [IPI-ROBO3.124] (Addgene#251721) shows significant binding only to human and mouse ROBO3. Each graph depicts the combined quantification of multiple images of the same transfected cells taken at 10X magnification. GFP or BFP-positive cells were identified via the neural network CellPose, then the mean fluorescence intensity (MFI) of the far-red channel for each cell, representing IPI-ROBO3.124 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 22000. IPI-ROBO3.124 staining of human and mouse variants of each ROBO family member is compared on the top and bottom rows. To test family-wide cross-reactivity, IPI-ROBO3.124 was used at 1 ug/mL (1:1000 dilution). doi: https://doi.org/10.57733/addgene.d4lio3

Immunohistochemistry (IHC)

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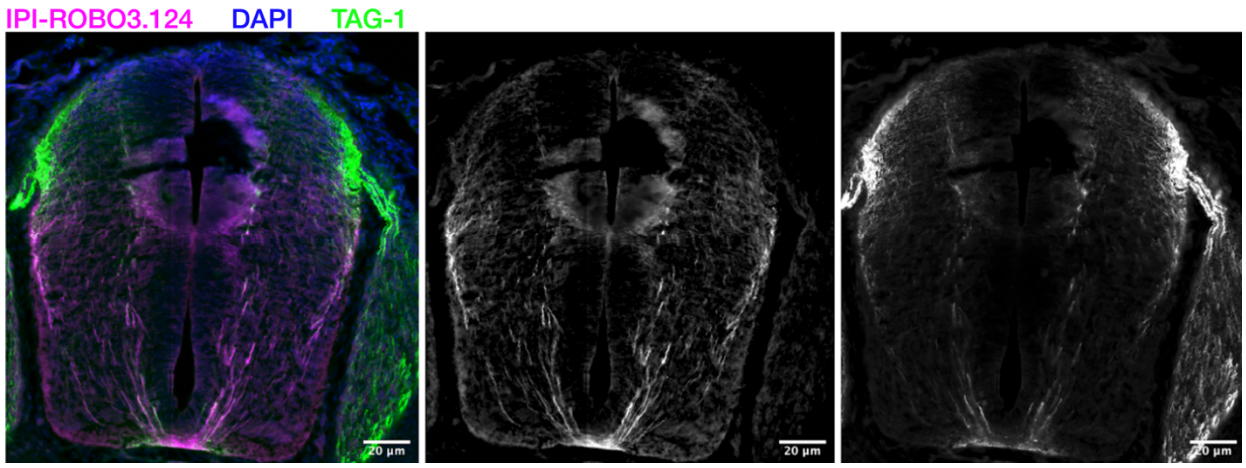


Anti-ROBO3 [IPI-ROBO3.124] (Addgene#251721) shows strongest staining along the commissural axons, particularly as they cross the floor plate, in accordance with extant literature describing the localization of ROBO3. Immunohistochemistry (IHC) of a 20 micron cryosection of the spinal cord of an E13 mouse embryo. Confocal images taken at 20X magnification on an ImageXpress confocal HT.ai microscope. Left image shows IPI-ROBO3.124 (magenta), commissural axon-labeling counterstain TAG1 (green) and DAPI (blue); the middle image shows only shows IPI-ROBO3.124, and the rightmost image only shows TAG1. IPI-ROBO3.124 was used at 10 ug/mL (1:100 dilution). doi: https://doi.org/10.57733/addgene.7js33w

Antibody Details

Antibody design and production

Human variable domains for the heavy and light chain of the FAB fragment used in yeast display were grafted onto the constant CH1, CH2 and CH3 domains of rabbit IgG. The chimera human/rabbit IgG1 construct was recombinantly expressed in Expi HEK293 cells, using pTipi2.1 as the expression vector. The antibody was purified by affinity chromatography using protein A (XYZ) and acid elution, followed by immediate buffer exchange using 1 x PBS buffer pH 7.4.

Sequence information

Heavy chain and light chain amino acid sequences are available upon request after purchase. **Contact us** to request.

Antibody Characterization

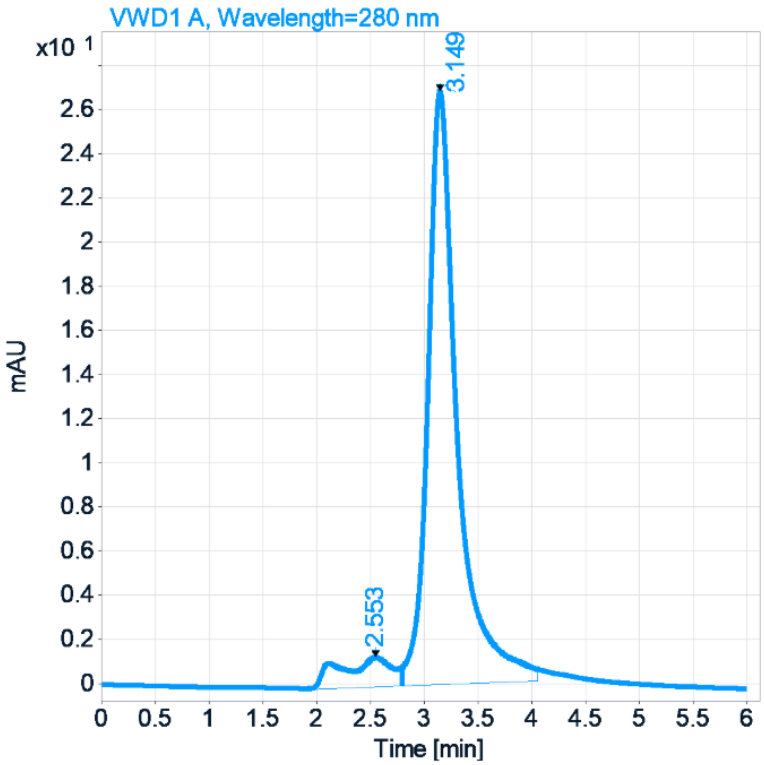
LC-MS: Intact mass analysis via LC-MS methods allows for confirmation antibody mass, and to identify any product-related variants such as glycosylation. Before conducting intact mass analysis via LC-MS, the antibody was reduced to its heavy chain (HC) and light chain (LC). This process allows for confirmation of the masses corresponding to the amino acid sequences of both chains.

	HC MW (Da) *Calculated*	HC MW (Da) *Observed*	HC MW (Da) Delta	LC MW (Da) *Calculated*	LC MW (Da) *Observed*	LC MW (Da) Delta
IPI-ROBO3.124	49711.16	49717.1	5.94	22952.39	22952.8	0.41

Heavy Chain (HC) Mass Calculation: The calculated molecular weight (MW) of the HC is derived by adding the mass of the unmodified HC amino acid sequence to the mass of the predominant N-glycan form (G0F), which is 1444.5 Da. This calculation assumes that the intrachain disulfide bonds remain intact. For HCs with an N-terminal glutamine (Q), the mass of Q is converted to pyroglutamic acid (PyroGlu), resulting in a deduction of 17.03 Da from the total mass. Additionally, for HCs with a C-terminal lysine (K), the mass of K (128.09 Da) is also subtracted.

Light Chain (LC) Mass Calculation: The calculated molecular weight (MW) of the LC is obtained from the mass of the unmodified LC amino acid sequence, assuming that the intrachain disulfide bonds are not reduced. For LCs with an N-terminal glutamine (Q), the mass of Q is converted to pyroglutamic acid (PyroGlu), leading to a deduction of 17.03 Da from the total mass. For LCs with a C-terminal lysine (K), the mass of K (128.09 Da) is subtracted as well.

Size Exclusion Chromatography (SEC): SEC is a protein purification technique that separates molecules based on size.



	RT (min)	Width (min)	Area	Height	Area %	Result
IPI-ROBO3.124	3.149	0.3166	510.7324	26.8856	91.8317	Pass

Antigen Details

Immunogen design:

cDNA of Human ROBO3 with C-terminal His-, Avi-, and HA-tags was produced in transiently transfected Expi293F cells and purified from culture supernatant by Ni-NTA affinity purification followed by size-exclusion chromatography.

Immunogen sequences:

>Human ROBO3 (AA: 21-891):
GDISNSSELLLGFNSSLAALNHTLLPPGDPSLNGSRVGPEDAMPRIVEQPPDLLVSRGEPATLPCRAEGR
PRPNIEWYKNGARVATVREDPRAHRLLLPSGALFFPRIVHGRRARPDEGVYTCVARNYLGAAASRNASL
EVAVLRDDFRQSPGNVVVAVGEPAVLECVPPRGHPEPSVSWRKDGARLKEEEGRITIRGGKLMMSHTLK
SDAGMYVCVASNMAGERESAAAEVMVLERPSFLRRPVNQVVLADAPVTFLCEVKGDPPPRLRWRKEDG
ELPTGRYEIRSDHSLWIGHVSAEDEGTYTCVAENSVGRAEASGSLSVHVPPQLVTQPQDQMAAPGESVA
FQCETKGNPPPAIFWQKEGSQVLLFPSQSLQPTGRFSVSPRGQLNITAVQRGDAGYYVCQAVSVAGSIL
AKALLEIKGASLDGLPPVILQGPANQTLVLGSSVWLPCRVTGNPQPSVRWKKDGQWLQGDDLQFKTMA
NGTLYIANVQEMDMGFYSCVAKSSTGEATWSGWLKMREDWGVSPDPPTEPSSPPGAPSQPVVTEITKN
SITLTWKPNPQTGAAVTSYVIEAFSPAAGNTWRTVADGVQLETHTVSGLQPNTIYLFLVRAVGAWGLSEP
SPVSEPVRTQDSSPSRPVEDPWRGQQGLAEVAVRLQEPIVLGPRTLQVSWTVDGPVQLVQGFRVSWRV
AGPEGGSWTMLDLQSPSQQSTVLRGLPPGTQIQIKVQAQGQEGLGAESLSVTRSIPEEAPSGPPQGVAV
ALGGDGNSSITVSWEPPLPSQQNGVITEYQIWCLGNESRFHLNRSAAGWARSAMLRGLVPGLLYRTLVA
AATSAGVGVPSAPVLVQLPSPPDLEPGLEVGAGLAVRLARVLREPGHHHHHHHHHHHGSGGLNDIFEAQ
KIEWHEGSGYPYDVPDYA

Sequence information:

HUGO:	13433
Uniprot:	Q96MS0
Refseq:	XM_024347470.1

Structural information:

Topology:	Single-pass type I membrane protein
PDB IDs:	6POG;6POK;6POL
Alphafold:	AF-Q96MS0-F1

Expression profiles:

Human Protein Atlas: ENSG00000154134

References

1. Z. Anderson, H. Li, T. Riedel, H. Zhu and D. Moshinsky. (2026). Flow cytometry for Anti-ROBO3 [IPI-ROBO3.124]. Addgene. https://doi.org/10.57733/addgene.vpcdbk
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3. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-ROBO3 [IPI-ROBO3.124]. Addgene. https://doi.org/10.57733/addgene.k6fbks
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5. A. Morano, T. Riedel, and D. Moshinsky. (2026). IHC for Anti-ROBO3 [IPI-ROBO3.124]. Addgene. https://doi.org/10.57733/addgene.7js33w

How to cite this antibody:
Anti-ROBO3 [IPI-ROBO3.124] - from Institute for Protein Innovation (IPI) (Addgene #251721; http://n2t.net/addgene:251721; RRID: AB_3720935).

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