# Product Datasheet

## Anti-ROBO1 [IPI-ROBO1.81]

Applications | Antibody Details | Antigen Details | References

### Overview

| | |
|---|---|
| Antigen | ROBO1 |
| Immunogen | Purified recombinant fragment of Human ROBO1, corresponding to AA: 26-898. |
| Host/isotype | Rabbit/IgG |
| Clonality | Recombinant monoclonal |
| Clone name | IPI-ROBO1.81 |
| RRID | AB_3720932 |
| IPI ID | TAB0010760-013-002 |
| Specificity | ROBO1; 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.3utuyc |
| IF – Binding | 1 µg/mL | Positive | https://doi.org/10.57733/addgene.6jpj14 |
| IF – Specificity | 1 µg/mL | Positive | https://doi.org/10.57733/addgene.3mvyvp |
| IHC | 10 µg/mL | Positive | https://doi.org/10.57733/addgene.m0y1uu |

‡ Not suitable for WB application.

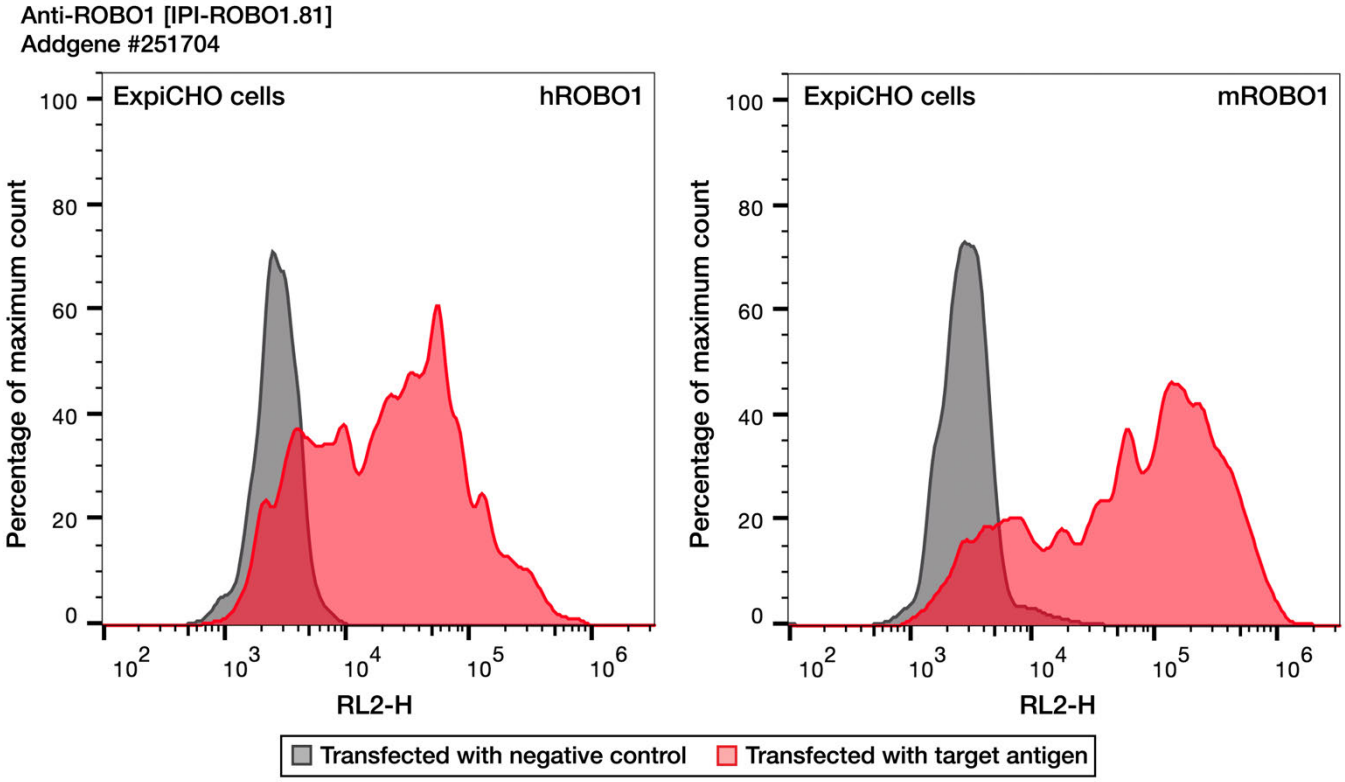
### Community Data*

| Application | Lab | Reference |
|---|---|---|
| IF | Homaira Nawabi, Ph.D., INSERM | https://doi.org/10.57733/addgene.z29s5s |
| IHC | Alain Chédotal, Ph.D., Institut de la Vision | https://doi.org/10.57733/addgene.42v0e6 |

* Supporting Data is generated by external partner labs, in the process of evaluating IPI antibody panels.

## Applications

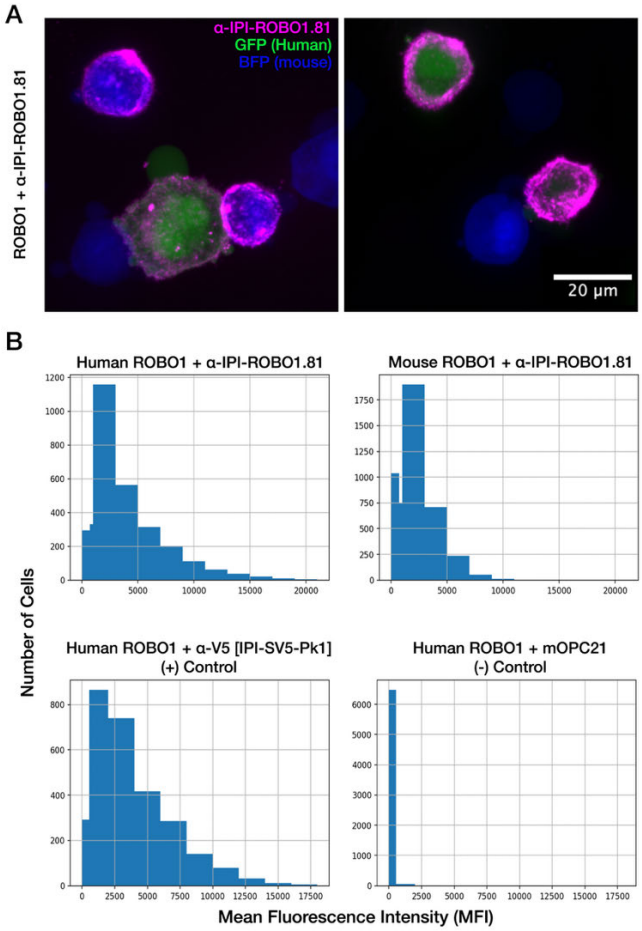
### Flow cytometry




**Anti-ROBO1 [IPI-ROBO1.81] (Addgene #251704) recognizes human and mouse ROBO1.** Histogram from FACS analysis on ExpiCHO cells transfected with human or mouse ROBO1 (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) ROBO1 were labeled with Anti-ROBO1 [IPI-ROBO1.81] 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.3utuyc

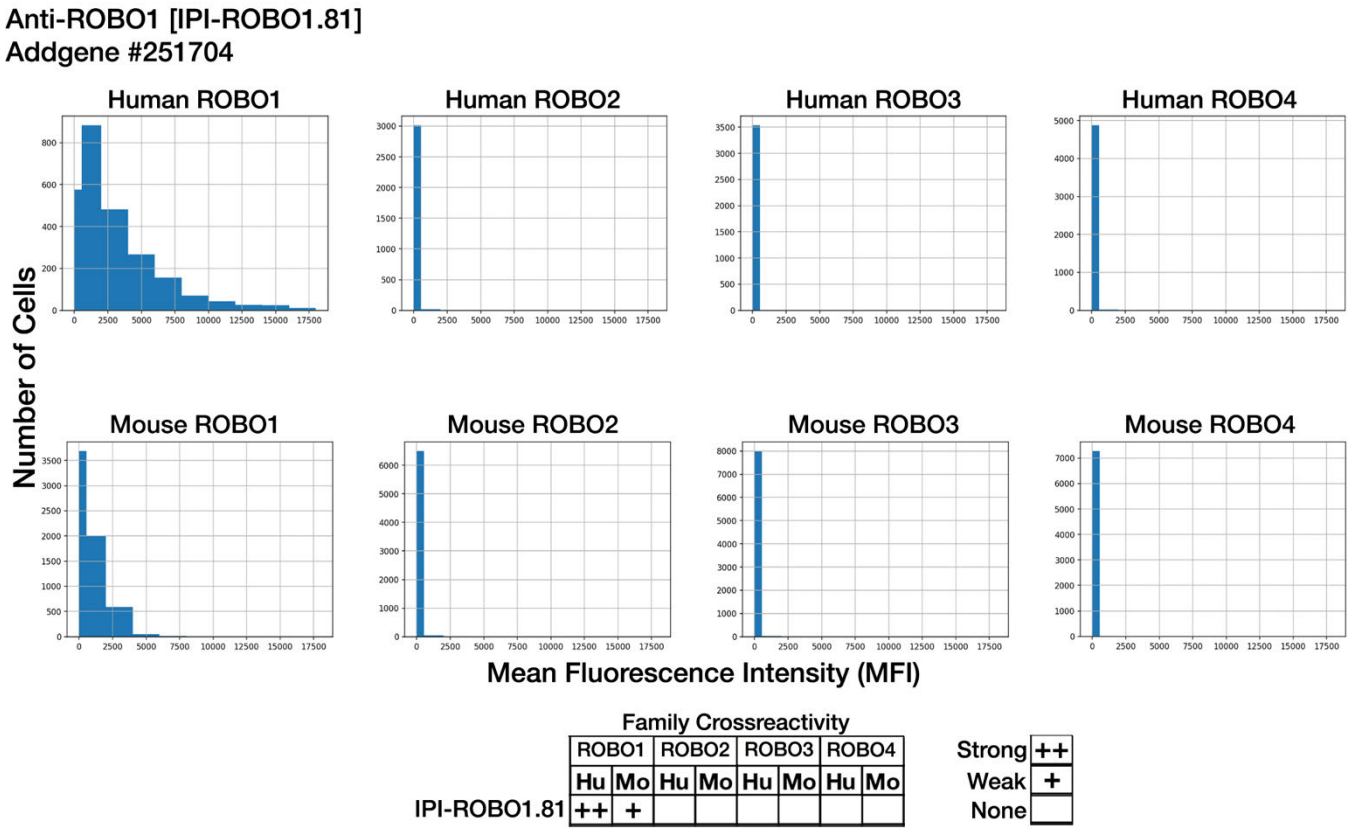
**$EC_{50}$ (data not shown):** A fourteen-point titration of antibody concentrations, ranging from 660 nM (0.1 mg/mL) to 4.42 pM with a 1:2.5 dilution factor, against human and mouse ROBO1 showed reactivity towards human and mouse ROBO1 with observed EC50 values of 0.48 nM (Human), 0.42 nM (Mouse).

## Immunofluorescence (IF) – Species Reactivity




**Anti-ROBO1 [IPI-ROBO1.81] (Addgene#251704) shows binding to human and mouse ROBO1.** A) Immunofluorescence (IF) of ExpiCHO cells transfected with human (Green/GFP) and mouse (Blue/BFP) ROBO1 stained with IPI-ROBO1.81 (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-ROBO1.81 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 22000. IPI-ROBO1.81 staining of human and mouse ROBO1 is shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-ROBO1.81 was used at 1 ug/mL (1:1,000 dilution). doi: https://doi.org/10.57733/addgene.6jpj14

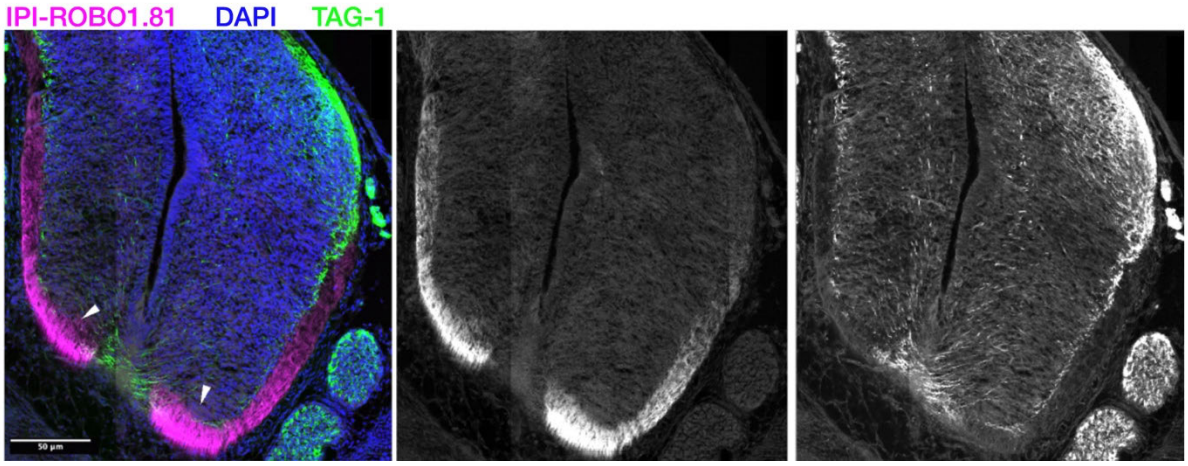
## Immunofluorescence (IF) – Target Specificity




**Anti-ROBO1 [IPI-ROBO1.81] (Addgene#251704) shows significant binding only to human and mouse ROBO1.** 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-ROBO1.81 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 22000. IPI-ROBO1.81 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-ROBO1.81 was used at 1 ug/mL (1:1000 dilution). doi: https://doi.org/10.57733/addgene.3mvyvp

## Immunohistochemistry (IHC)

Anti-ROBO1 [IPI-ROBO1.81]
Addgene #251704




**Anti-ROBO1 [IPI-ROBO1.81] (Addgene#251704) shows strongest staining along the ventral funiculus (white arrows), in accordance with extant literature describing the localization of ROBO1.**
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-ROBO1.81 (magenta), commissural axon-labeling counterstain TAG1 (green) and DAPI (blue); the middle image shows only shows IPI-ROBO1.81, and the rightmost image only shows TAG1. IPI-ROBO1.81 was used at 10 ug/mL (1:100 dilution). doi: https://doi.org/10.57733/addgene.m0y1uu

## 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

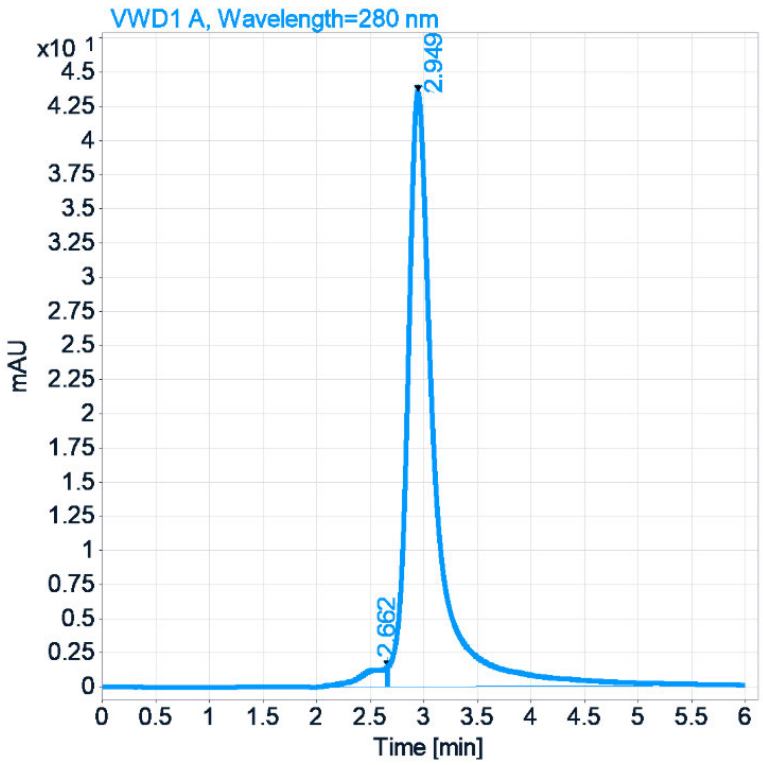
**LC-MS:** Intact mass analysis via LC-MS methods allows for confirmation antibody mass, and to identify any product-related variants such as glycosylation. Before conducting intact mass analysis via LC-MS, the antibody was reduced to its heavy chain (HC) and light chain (LC). This process allows for 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-ROBO1.81** | 49484.85 | 49491.16 | 6.31 | 22618.94 | 22618.39 | -0.55 |

**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-ROBO1.81** | 2.949 | 0.2951 | 770.6906 | 43.528 | 96.9986 | Pass |

## Antigen Details

**Immunogen design:**

cDNA of Human ROBO1 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 ROBO1 (AA: 26-898):
QLIPDPEDVERGNDHGTPIPTSDNDDNSLGYTGSRLRQEDFPPRIVEHPSDLIVSKGEPATLNCKAEGRP
TPTIEWYKGGERVETDKDDPRSHRMLLPSGSLFFLRIVHGRKSRPDEGVYVCVARNYLGEAVSHNASLE
VAILRDDFRQNPSDVMVAVGEPAVMECQPPRGHPEPTISWKKDGSPLDDKDERITIRGGKLMITYTRKSD
AGKYYVCVGTNMVGERESEVAELTVLERPSFVKRPSNLAVTVDDSAEFKCEARGDPVPTVRWRKDDGELP
KSRYEIRDDHTLKIRKVTAGDMGSYTCVAENMVGKAEASATLTVQEPPHFVVKPRDQVVALGRTVTFQCE
ATGNPQPAIFWRREGSQNLLFSYQPPQSSSRFSVSQTGDLTITNVQRSDVGYYICQTLNVAGSIITKAYLE
VTDVIADRPPPVIRQGPVNQTVAVDGTFVLSCVATGSPVPTILWRKDGVLVSTQDSRIKQLENGVLQIRYA
KLGDTGRYTCIASTPSGEATWSAYIEVQEFGVPVQPPRPTDPNLIPSAPSKPEVTDVSRNTVTLSWQPNL
NSGATPTSYIIEAFSHASGSSWQTVAENVKTETSAIKGLKPNAIYLFLVRAANAYGISDPSQISDPVKTQDV
LPTSQGVDHKQVQRELGNAVLHLHNPTVLSSSSIEVHWTVDQQSQYIQGYKILYRPSGANHGESDWLVF
EVRTPAKNSVVIPDLRKGVNYEIKARPFFNEFQGADSEIKFAKTLEEAPSAPPQGVTVSKNDGNGTAILVS
WQPPPEDTQNGMVQEYKVWCLGNETRYHINKTVDGSTFSVVIPFLVPGIRYSVEVAASTGAGSGVKSEP
QFIQLDAHGNPVSPEDQVSLAQQISDVVKQPGHHHHHHHHHHHGSGGLNDIFEAQKIEWHEGSGYPYDV
PDYA

**Sequence information:**

HUGO: 10249
Uniprot: Q9Y6N7
Refseq: XM_047448661.1

**Structural information:**

Topology: Single-pass type I membrane protein
PDB IDs: 2EO9;2V9Q;2V9R;2V9T;3WIH;4HLJ;5O5G;5O5I;5OPE;6A77;6A78;6A79
Alphafold: AF-Q9Y6N7-F1

**Expression profiles:**

Human Protein Atlas: ENSG00000169855

## References

1. Z. Anderson, H. Li, T. Riedel, H. Zhu and D. Moshinsky. (2026). Flow cytometry for Anti-ROBO1 [IPI-ROBO1.81]. Addgene. https://doi.org/10.57733/addgene.3utuyc
2. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-ROBO1 [IPI-ROBO1.81]. Addgene. https://doi.org/10.57733/addgene.6jpj14
3. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-ROBO1 [IPI-ROBO1.81]. Addgene. https://doi.org/10.57733/addgene.3mvyvp
4. A. Morano, T. Riedel, and D. Moshinsky. (2026). IHC for Anti-ROBO1 [IPI-ROBO1.81]. Addgene. https://doi.org/10.57733/addgene.m0y1uu
5. Y. Zagar and A. Chédotal. (2026). IHC for Anti-ROBO1 [IPI-ROBO1.81]. Addgene. https://doi.org/10.57733/addgene.42v0e6
6. A. Saintpierre and H. Nawabi. (2026). ICC/IF for Anti-ROBO1 [IPI-ROBO1.81]. Addgene. https://doi.org/10.57733/addgene.89j4uw

**How to cite this antibody:**
Anti-ROBO1 [IPI-ROBO1.81] - from Institute for Protein Innovation (IPI) (Addgene #251704; http://n2t.net/addgene:251704; RRID: AB_3720932).

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