Product Datasheet

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Overview

Antigen	SEMA5B
Immunogen	Purified recombinant fragment of Mouse SEMA5B, corresponding to AA: 27 - 573).
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-mSEM5B.2
RRID	AB_3751924
IPI ID	TAB0012940-013
Specificity	SEMA5B; Dose not cross-react with other SEMA5s.
Species	human and mouse
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 +4 °C
Storage	Store at +4C for up to 3 months. For long-term storage, aliquot and store at -20C. Avoid multiple freeze/thaw cycles.

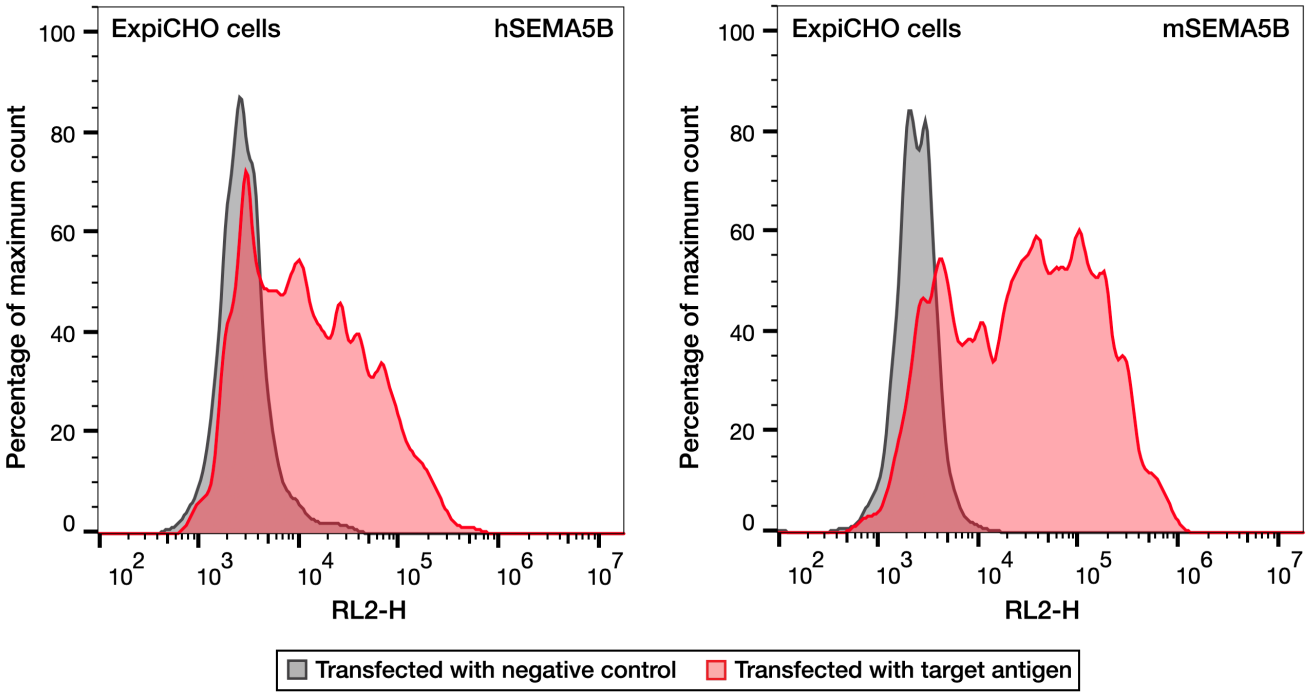
IPI Tested Applications ‡

Applications	Tested Concentration	Result	Reference
Flow Cytometry	0.66-100 µg/mL	Positive	https://doi.org/10.57733/addgene.9lwar6
IF- Binding	0.1 µg/mL	Positive	https://doi.org/10.57733/addgene.rzyzhe
IF - Specificity	0.1 µg/mL	Positive	https://doi.org/10.57733/addgene.9midfo
IHC	10 µg/mL	Positive	https://doi.org/10.57733/addgene.55hpi8

‡ Not suitable for WB application.

Applications

Cell Display

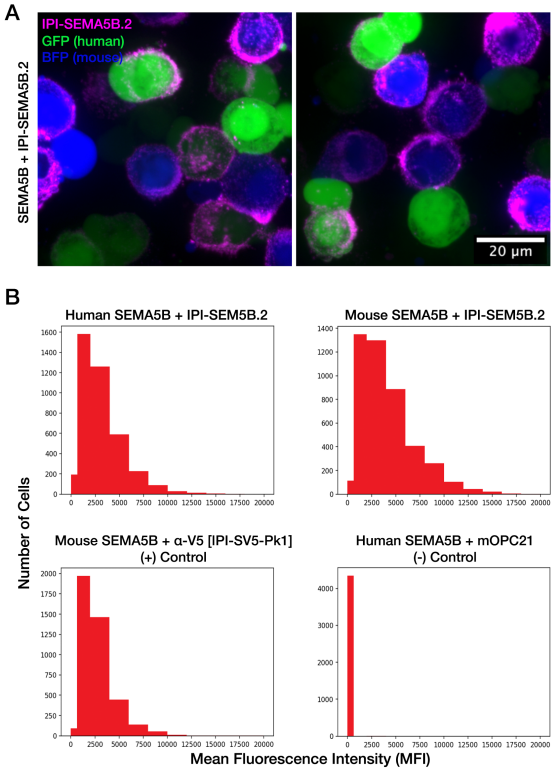


Anti-SEMA5B [IPI-mSEM5B.2] (Addgene #259422) recognizes human and mouse SEMA5B.

Histogram from FACS analysis on ExpiCHO cells transfected with human or mouse SEMA5B (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) SEMA5B were labeled with Anti-SEMA5B [IPI-mSEM5B.2] 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.9lwar6

Immunofluorescence (IF) – Binding

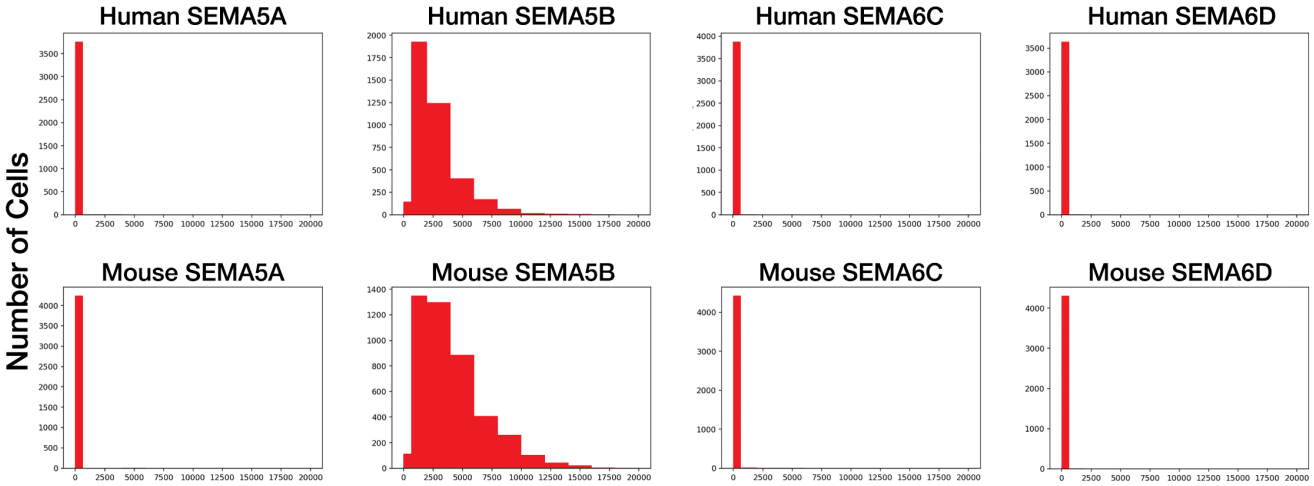


Anti-SEMA5B [IPI-mSEM5B.2] (Addgene#259422) shows binding to human and mouse SEMA5B.

A) Immunofluorescence (IF) of ExpiCHO cells transfected with human (Green/GFP) and mouse (Blue/BFP) NRP1 stained with IPI-mSEM5B.2 (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-mNRP1.23 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 20000. IPI-mSEM5B.2 staining of human and mouse SEM5B is shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-mSEM5B.2 was used at 0.1 ug/mL (1:10,000 dilution).

doi: https://doi.org/10.57733/addgene.rzyzhe

Immunofluorescence (IF) - Target Specificity



Family Crossreactivity

	SEM5A		SEM5B		SEM6C		SEM6D	
	Hu	Mo	Hu	Mo	Hu	Mo	Hu	Mo
IPI-mSEM5B.2			++	++				

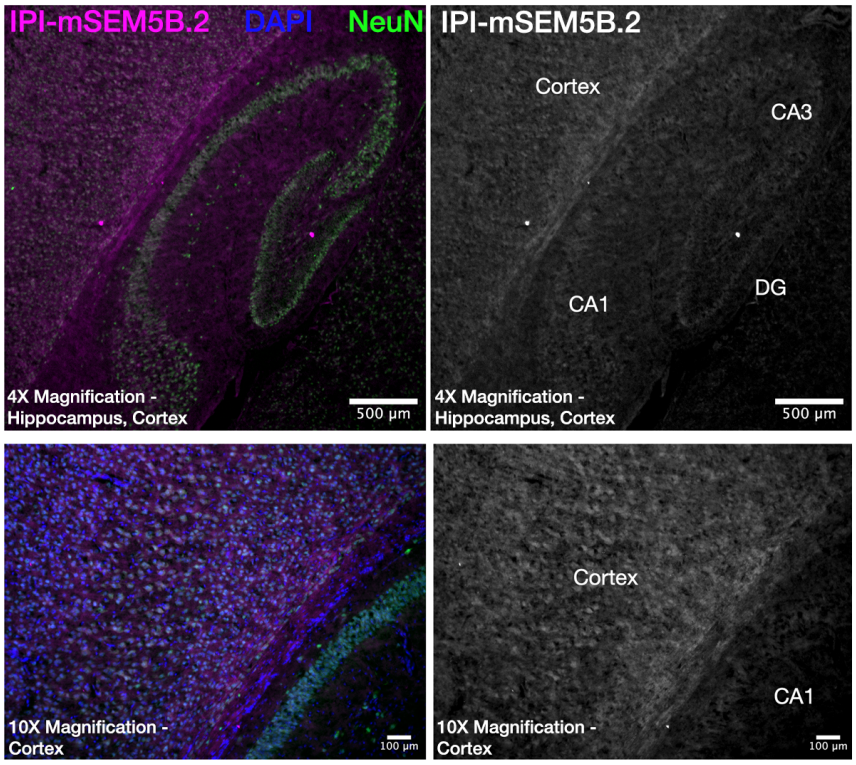
Strong	++
Weak	+
None	

Anti-SEMA5B [IPI-mSEM5B.2] (Addgene#259422) shows significant binding only to human and mouse SEMA5B.

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-mSEM5B.2 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 20000. IPI-mSEM5B.2 staining of human and mouse variants of NRP family members is compared on the top and bottom rows. To test family-wide cross-reactivity, IPI-mSEM5B.2 was used at 0.1 ug/mL (1:10000 dilution).

doi: https://doi.org/10.57733/addgene.9midfo

Immunohistochemistry (IHC)



Immunohistochemistry (IHC) for Anti-SEMA5B [IPI-mSEM5B.2]

Immunohistochemistry (IHC) of a 20 micron cryosection of the hippocampus of an immature (P10) mouse brain. Widefield images taken at 4X (Top row) and 10X (bottom row) magnification on an EVOS m7000 microscope. Left image shows IPI-mSEM5B.2 (magenta) and neuronal cell body labeling counterstain NeuN (green); on the bottom row, nuclear stain DAPI (blue) is alsp present in the left image. For both rows, the right image shows only IPI-mSEM5B.2. IPI-mSEM5B.2 was used at 10 ug/mL (1:100 dilution).

doi: https://doi.org/10.57733/addgene.55hpi8

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 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

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.

LC-MS Results

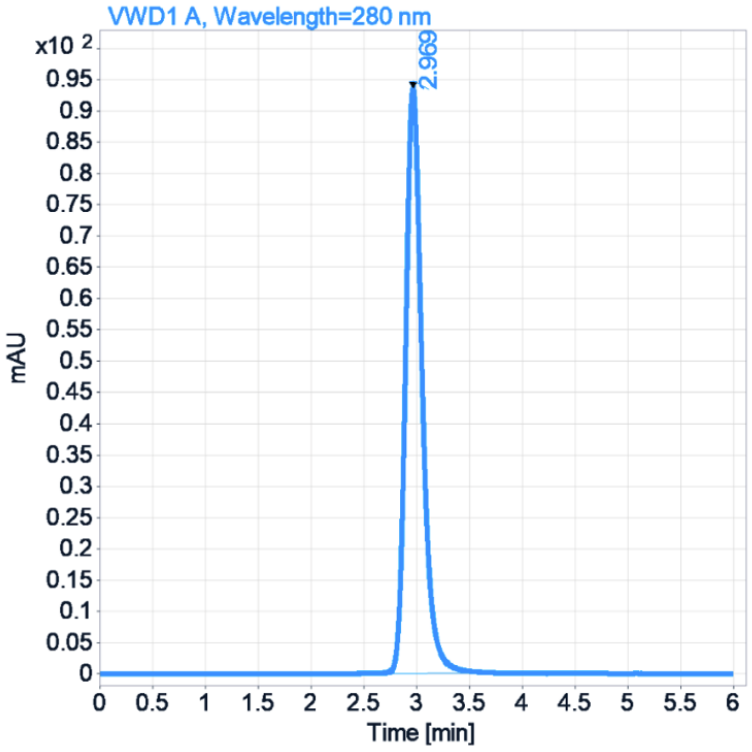
LC Calculated	LC Observed	LC Delta	HC Calculated	HC Observed	HC Delta
22619.02	22618.74	-0.28	50237.71	50243.60	5.89

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 Cterminal 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)

5 µL of 1±0.2 mg/mL of sample was analyzed with TSKgel® SuperSW mAb HTP (4 µm) HPLC Column in 1X PBS, pH 7.4 as the mobile phase.



Clone	RT (min)	Width (min)	Area	Height	Area %	Result
IPI-mSEM5B.2	2.97	0.17	1022.28	93.52	100.00	Pass

Interpretation: SEC-QC pass. Main peak with greater than 90% purity and within retention time of 2.6 to 3.26 mins.

Antigen Details

Immunogen sequence : Mouse SEMA5B (AA: 27 - 573):

QDIASESSSEQQMCTRREHPIVAFEDLKPWVFNFTYPGVRDFSQLALDPSRNQLIVGARNYLFRLSLANVSLLQATEWAS
SEDTRRSCQSKGKTEEECQNYVRVLIVSGRKVFMCGTNAFSPVCSSRQVGNLSRTIEKINGVARCPYDPRHNSTAVISS
QGELYAATVIDFSGRDPAIYRSLGSGPPLRTAQYNSKWLNEPNFVAAFDIGLFAYFFLRENAVEHDCGRTVYSRVARVCK
NDVGGRFLLEDTWTTFMKARLNCSRPGEVPFYYNELQSAFHLPEQDLIYGVFTTNVNSIAASAVCAFNLSAISKAFNGPF
RYQENPRAAWLPIANPIPNFQCGTLPETGPNENLTERSLQDAQRLFLMSEAVQPVTPEPCVTQDSVRFSHLVVDLVQAK
DTLYHVLYIGTESGTILKALSTASRSLRGCYLEELHVLPPGRLEPLRSLRILHSARALFVGLSDRVLRVPLERCSAYHSQGA
CLGARDPYCGWDGKRQLCSTLEDSSNMSLWIQNITTCPVRNVTGGGGSGGGGSDYKDDDDKGSGGLNDIFEAQKIEW
HEGSGHHHHHHHH

Sequence information:

HUGO: MGI:107555
Uniprot: Q60519
Refseq: NM_013661.3

Structural information:

Topology: Single-pass type I membrane protein
PDB IDs: -
Alphafold: AF-Q60519-F1

Expression profile:

Human Protein Atlas: ENSG00000082684

References

1. Z. Anderson, H. Li, T. Riedel, H. Zhu and D. Moshinsky. (2026). Flow for Anti-SEMA5B [IPI-mSEM5B.2]. Addgene. https://doi.org/10.57733/addgene.9lwar6
2. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC for Anti-SEMA5B [IPI-mSEM5B.2]. Addgene. https://doi.org/10.57733/addgene.rzyzhe
3. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC for Anti-SEMA5B [IPI-mSEM5B.2]. Addgene. https://doi.org/10.57733/addgene.9midfo
4. A. Morano, T. Riedel, and D. Moshinsky. (2026). IHC for Anti-SEMA5B [IPI-mSEM5B.2]. Addgene. https://doi.org/10.57733/addgene.55hpi8

How to cite this antibody:

Anti-SEMA5B [IPI-mSEM5B.2] - from Institute for Protein Innovation (IPI) (Addgene #259422; https://n2t.net/addgene:259422; RRID: AB_3751924).

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