Product Datasheet

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Overview

Antigen	SEMA3D
Immunogen	Purified recombinant fragment of Human, SEMA3D, corresponding to AA: 38 - 588.
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-SEM3D.2
RRID	AB_3740896
IPI ID	TAB0012517-013
Specificity	SEMA3D; Does not cross-react with other SEMAs.
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.

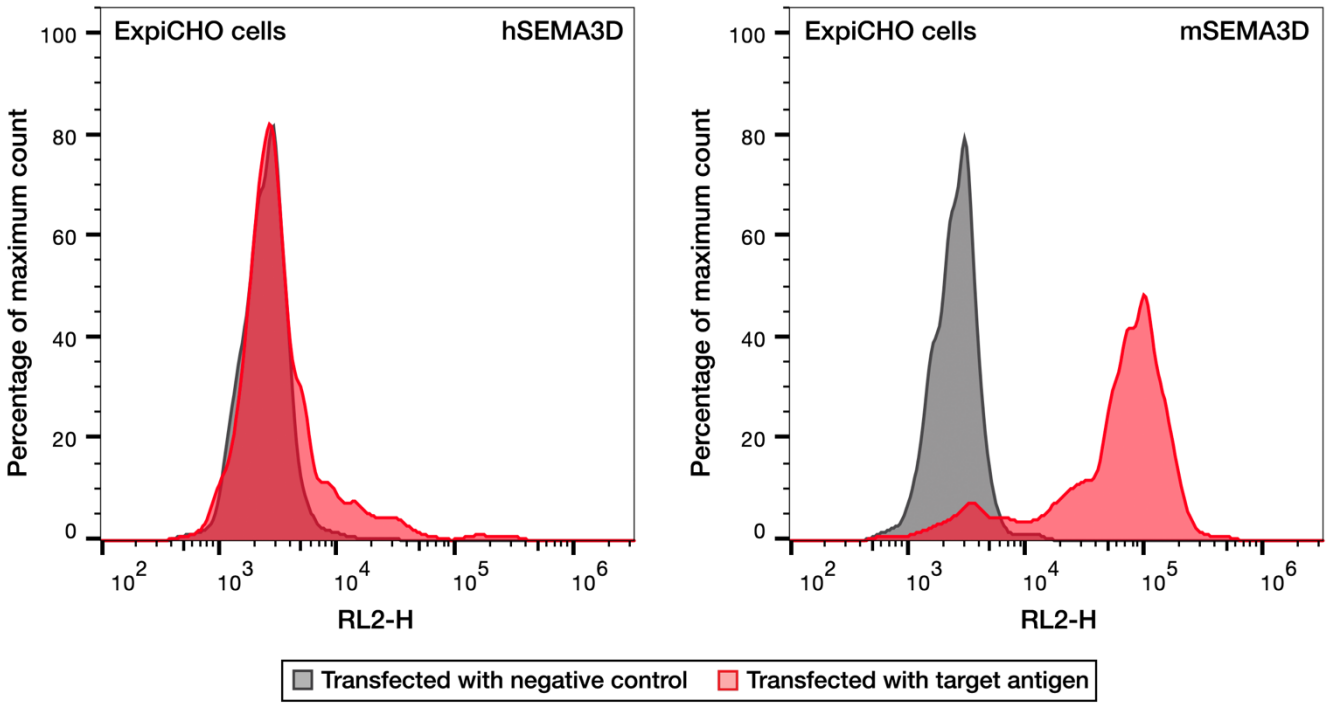
IPI Tested Applications‡

Application	Tested concentration	Result	Reference
Flow	0.66-100 µg/mL	Positive	https://doi.org/10.57733/addgene.3qt9q9
IF – Binding	10 µg/mL (1:100)	Positive	https://doi.org/10.57733/addgene.wvy8ki
IF – Specificity	10 µg/mL (1:100)	Positive	https://doi.org/10.57733/addgene.y27uj9

‡ Not suitable for WB application.

Applications

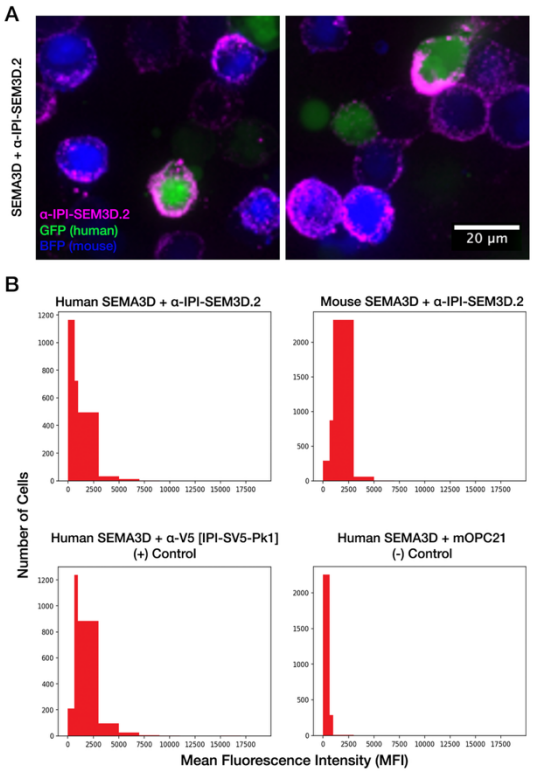
Flow cytometry



Anti-SEMA3D [IPI-SEM3D.2] (Addgene #254648) recognizes human and mouse SEMA3D. Histogram from FACS analysis on ExpiCHO cells transfected with human or mouse SEMA3D (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) SEMA3D were labeled with Anti-SEMA3D [IPI-SEM3D.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.https://doi.org/10.57733/addgene.3qt9q9

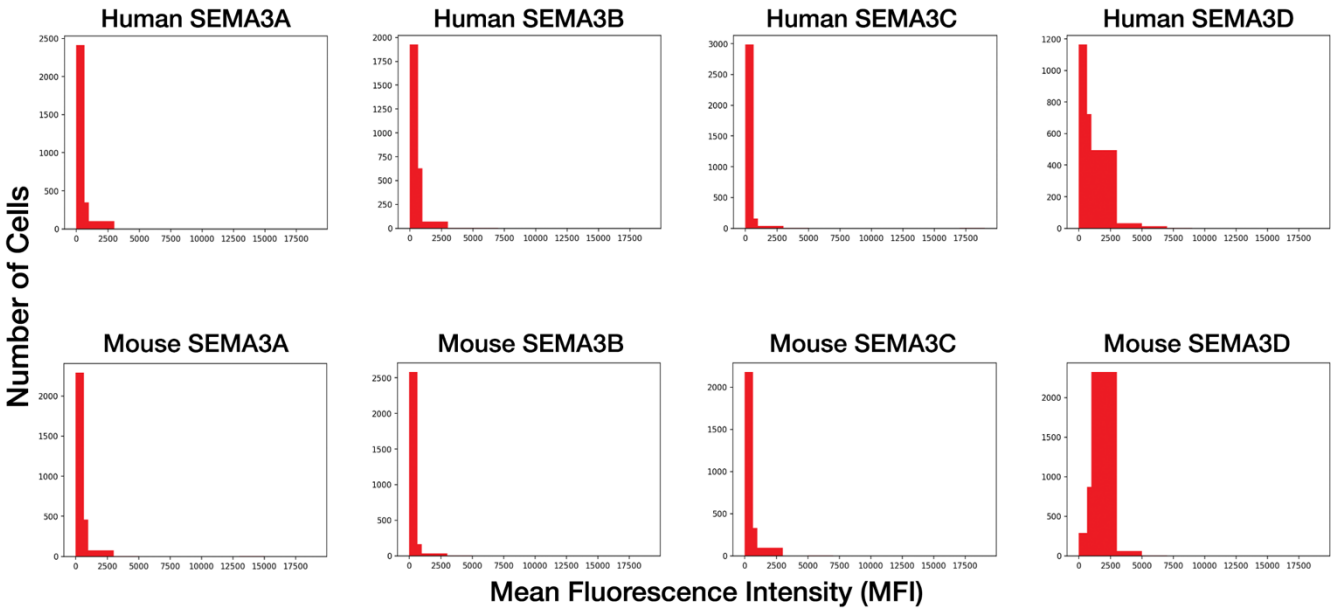
EC_{50} (data not shown): A fourteen-point titration of antibody concentrations, ranging from 660 nM (0.1 mg/mL) to 4.42 pM with a 1:2.5 dilution factor, against human and mouse SEMA3D showed reactivity towards mouse SEMA3D with observed EC50 values of 3.88 nM (mouse).

Immunofluorescence (IF) – Species Reactivity



Anti-SEMA3D [IPI-SEM3D.2] (Addgene#254648) shows binding to human and mouse SEM3D. A) Immunofluorescence (IF) of ExpiCHO cells transfected with human (Green/GFP) and mouse (Blue/BFP) SEMA3D stained with IPI-SEM3D.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-SEM3D.2 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 20000. IPI-SEM3D.2 staining of human and mouse SEM3D is shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-SEM3D.2 was used at 10 ug/mL (1:100 dilution).
https://doi.org/10.57733/addgene.wvy8ki

Immunofluorescence (IF) – Target Specificity



Family Crossreactivity

	SEM3A		SEM3B		SEM3C		SEM3D	
	Hu	Mo	Hu	Mo	Hu	Mo	Hu	Mo
IPI-SEM3D.2							++	++

Strong ++
Weak +
None

Anti-SEMA3D [IPI-SEM3D.2] (Addgene#254648) shows significant binding only to human and mouse SEM3D. 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-SEM3D.2 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 20000. IPI-SEM3D.2 staining of human and mouse variants of selected SEMA3 family members is compared on the top and bottom rows. To test family-wide cross-reactivity, IPI-SEM3D.2 was used at 10 ug/mL (1:100 dilution). https://doi.org/10.57733/addgene.y27uj9

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

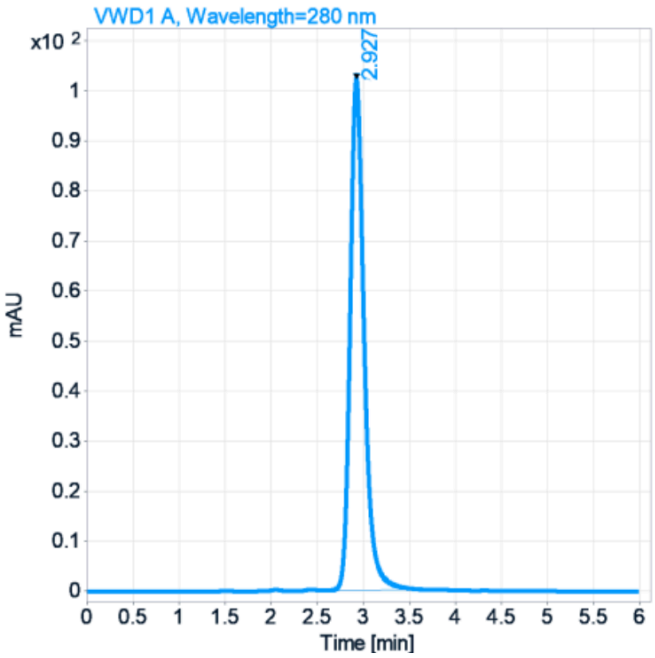
LC-MS: Intact mass analysis via LC-MS methods allows for confirmation antibody mass, and to identify any product-related variants such as glycosylation. Before conducting intact mass analysis via LC-MS, the antibody was reduced to its heavy chain (HC) and light chain (LC). This process allows for confirmation 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-SEM3D.2	49782.27	49788.86	6.59	22952.44	22952.00	-0.44

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): 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.



	RT (min)	Width (min)	Area	Height	Area %	Result
IPI-SEM3D.2	2.93	0.16	1082.05	102.15	100	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 design:

cDNA of Human SEMA3D with C-terminal His-, and FLAG-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 SEMA3D (AA: 38 - 588):
SKQNIPRLKLTYKDLLLSNTCIPFLGSSEGLDFQTLLLDEERGILLLGAKDHVFLLSLVDLNKNFKKIYWPA
AKERVELCKLAGKDANAECANFIRVLQPYNKTHVYVCGTGAFHPLCGYIDLGANKEELIFKLDTHNLESG
RLKCPFDPQQPFASVMTDEHLYSGTASDFLGKDTAFTRSLGLMQDHHSIRTDISEHHWLNGAKFIGTFPI
PDTYNPDDDKIYFFFRESSQEGSTSDRSILSRVGRVCKNDVGGQRSLINKWTTFLKARLICSIPGSDGADT
HFDELQDIYLLPTRDERNPVVYGVFTTTSSIFKGSAVCVYSMADIRAVFNGPYAHKESADHRWVQYDGRI
PYPRPGTCPSKTYDPLIKSTRDFPDDVISFIRRHPVMYKSVYPVAGAPTFKRINVDYRLTQIVVDHVVAEDG
QYDVMFLGTDIGTVLKVVSISKEKWNMEEVVLEELQVFKHPTAILNMELSLKQQQLYVGSWDGLVQLSLH
RCDTYGKACADCCLARDPYCAWDGNACSRYAPTSKSRASRQDVKYGDPITQCWDIEGGGGSGGGGS
DYKDDDDKGSGGLNDIFEAQKIEWHEGSGHHHHHHHH

Sequence information:

HUGO:	MGI:1860118
Uniprot:	Q8BH34
Refseq:	NM_028882.4

Structural information:

Topology:	Secreted extracellular protein
PDB IDs:	-
Alphafold:	AF-Q8BH34-F1

Expression profiles:

Human Protein Atlas: ENSMUSG00000040254

References

1. Z. Anderson, H. Li, T. Riedel, H. Zhu and D. Moshinsky. (2026). Flow Cytometry for Anti-SEMA3D [IPI-SEM3D.2]. Addgene. https://doi.org/10.57733/addgene.3qt9q9
2. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-SEMA3D [IPI-SEM3D.2] in binding assay. Addgene. https://doi.org/10.57733/addgene.wvy8ki
3. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-SEMA3D [IPI-SEM3D.2] in specificity assay. Addgene. https://doi.org/10.57733/addgene.y27uj9

How to cite this antibody:
Anti-SEMA3D [IPI-SEM3D.2] - from Institute for Protein Innovation (IPI) (Addgene #254648; http://n2t.net/addgene:254648; RRID: AB_3740896).

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