Product Datasheet

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Overview

Antigen	SEMA6A
Immunogen	Purified recombinant fragment of mouse SEMA6A, corresponding to AA: (19-628).
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-mSEM6A.18
RRID	AB_3741663
IPI ID	TAB0012597-013
Specificity	SEMA6A; Does not cross-react with other SEMA6s.
Species	human (weak) 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.

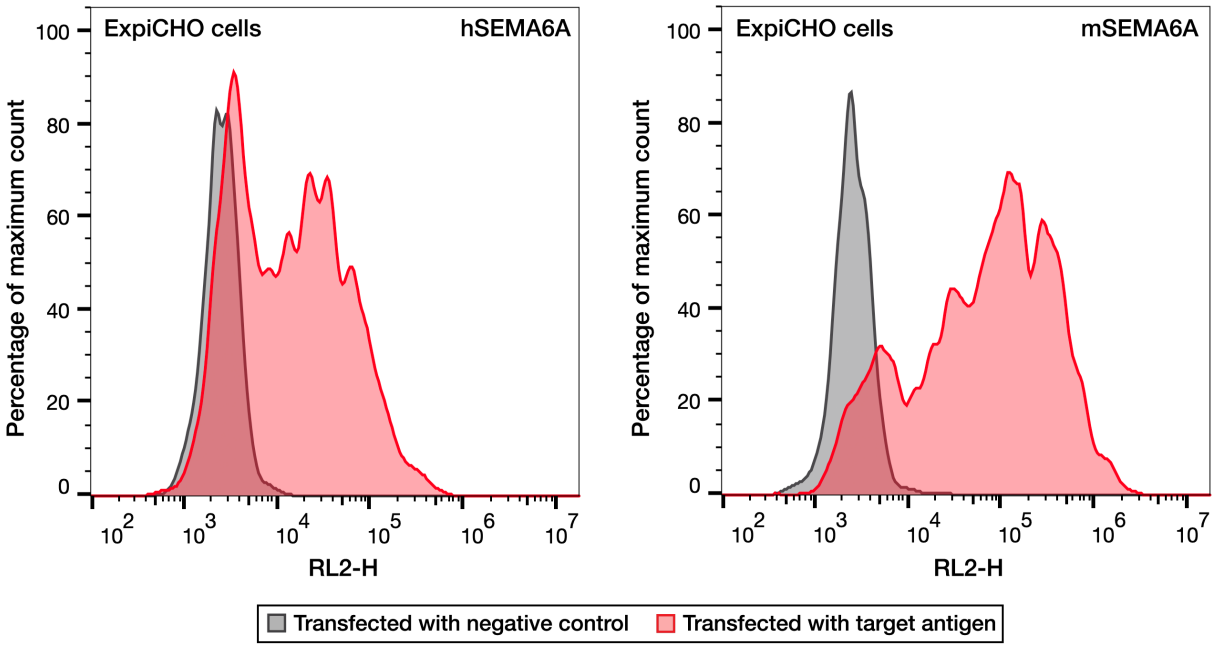
IPI Tested Applications ‡

Applications	Tested Concentration	Result	Reference
Flow	0.66-100 µg/mL	Positive	https://doi.org/10.57733/addgene.285lqs
IF - Binding	1 µg/mL	Positive	https://doi.org/10.57733/addgene.l4yumk
IF - Specificity	1 µg/mL	Positive	https://doi.org/10.57733/addgene.ndwxet

‡ Not suitable for WB application.

Applications

Cell Display

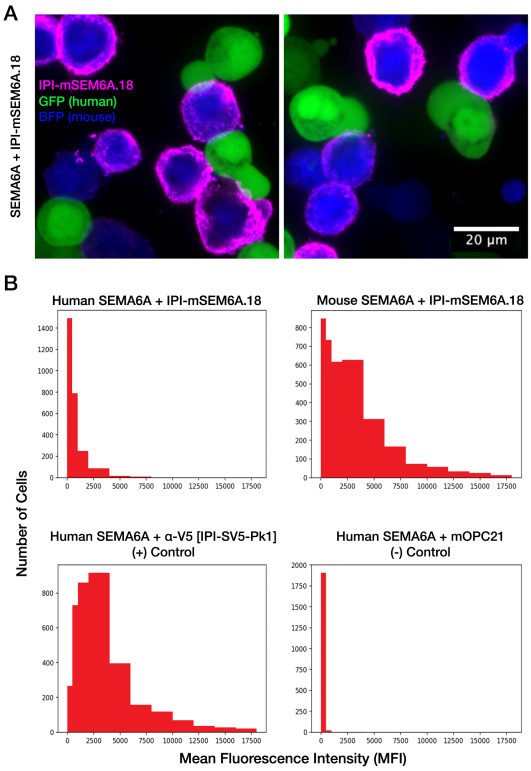


Anti-SEMA6A [IPI-mSEM6A.18] (Addgene #255663) recognizes human (weak) and mouse SEMA6A.

Histogram from FACS analysis on ExpiCHO cells transfected with human or mouse SEMA6A (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) SEMA6A were labeled with Anti-SEMA6A [IPI-mSEM6A.18] 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.285lqs

Immunofluorescence (IF) – Binding

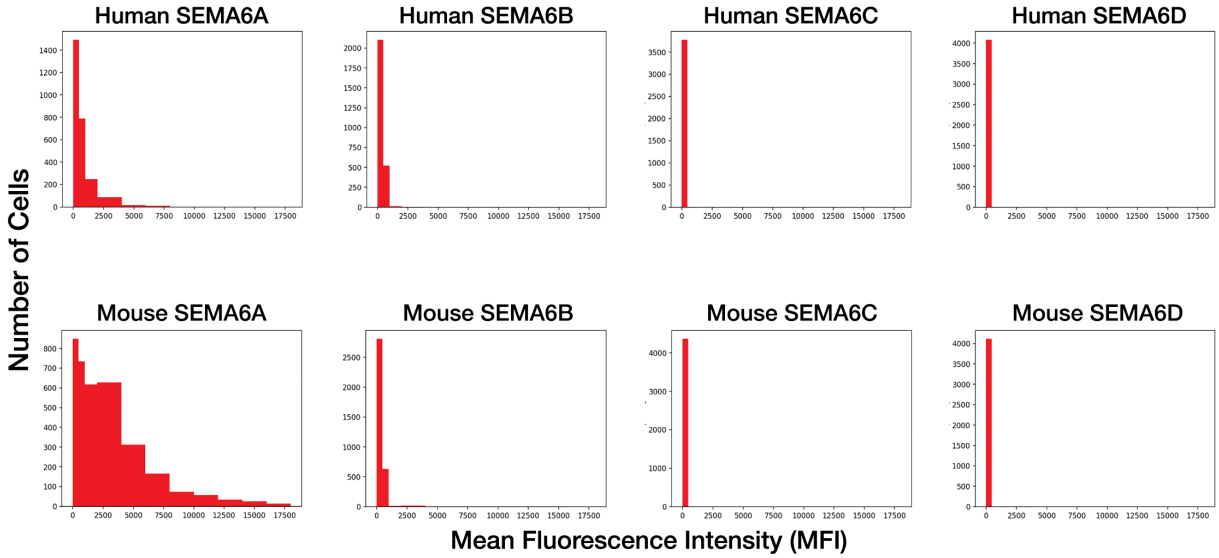


Anti-SEMA6A [IPI-mSEM6A.18] (Addgene#255663) shows binding to mouse SEM6A with weak binding to human SEM6A.

A) Immunofluorescence (IF) of ExpiCHO cells transfected with human (Green/GFP) and mouse (Blue/BFP) SEM6A stained with IPI-mSEM6A.18 (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-mSEM6A.18 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 20000. IPI-mSEM6A.18 staining of human and mouse SEM6A is shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-mSEM6A.18 was used at 0.1 ug/mL (1:10000 dilution).

doi: https://doi.org/10.57733/addgene.l4yumk

Immunofluorescence (IF) - Target Specificity



Family Crossreactivity

	SEM6A		SEM6B		SEM6C		SEM6D	
	Hu	Mo	Hu	Mo	Hu	Mo	Hu	Mo
IPI-SEM6A.18	++	++						

Strong	++
Weak	+
None	

Anti-SEMA6A [IPI-mSEM6A.18] (Addgene#255663) shows significant binding only to human and mouse SEM6A.

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-mSEM6A.18 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 20000. IPI-mSEM6A.18 staining of human and mouse variants of selected SEMA6 family members is compared on the top and bottom rows. To test family-wide cross-reactivity, IPI-mSEM6A.18 was used at 1 ug/mL (1:1000 dilution).

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

Antibody Details

Antibody Design and Production

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

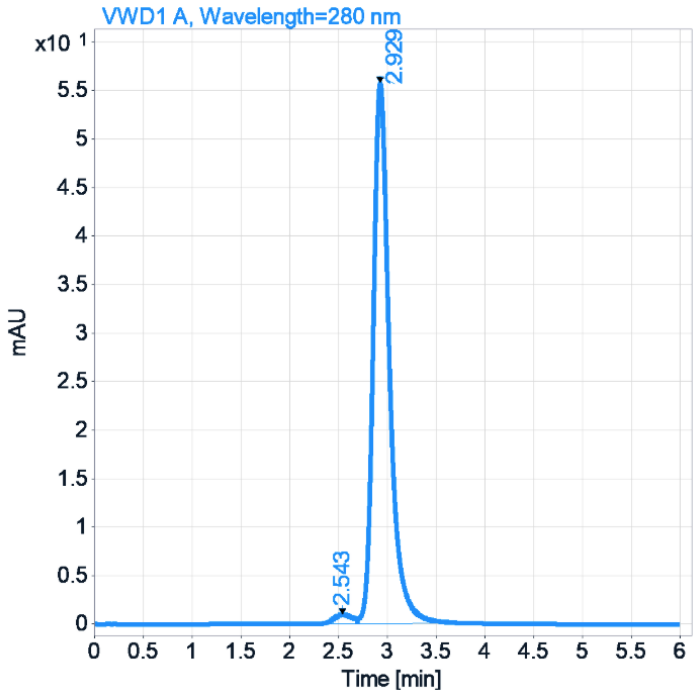
LC Calculated	LC Observed	LC Delta	HC Calculated	HC Observed	HC Delta
22619.02	22618.47	-0.55	49800.24	49806.00	5.76

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-mSEM6A.18	2.93	0.19	645.36	55.75	98.04	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

>MouseSEMA6A(AA:19-628):GFPEDSEPISISHGNYTKQYPVFVGHKPGRNTTQRHRLDIQMIMIMNRTLYVAA
RDHIYTVDIDTSHTEEIYCSKKLTWKSRQADVDTCRMKGKHKDECHNFIKVLLKKNDDTLFVCGTNAFNPSCRN
YRVDTLETFGDEFSGMARCPYDAKHANIALFADGKLYSATVTDFLAIDAVIYRSLGDSPTLRTVKHDSKWLKEPY
FVQAVDYGDYIYFFFREIAVEYNTMGKVVFPRVAQVCKNDMGGSQRVLEKQWTSFLKARLNCSVPGDSHFYFN
ILQAVTDVIRINGRDVVLATFSTPYNSIPGSAVCAYDMLDIANVFTGRFKEQKSPDSTWTPVPDERVPKPRPGCC
AGSSSLEKYATSNEFPDDTLNFIKTHPLMDEAVPSIINRPWFLRTMVRYRLTKIAVDNAAGPYQNHTVVFLGSEK
GIILKFLARIGSSGFLNGSLFLEEMNVYNPEKCSYDGVEDKRIMGMQLDRASGSLYVAFSTCVIKVPLGRCERHG
KCKKTCIASRDPYCGWVRESGSCAHLSPLSRLTFEQDIERGNTDGLGDCHNSGGGGSGGGGSDYKDDDDKG
SGGLNDIFEAQKIEWHEGSGHHHHHHHH

Sequence information:

HUGO:	MGI:1203727
Uniprot:	O35464
Refseq:	XP_006525789

Structural information:

Topology:	Single-pass type I membrane protein
PDB IDs:	6WTS
Alphafold:	AF-O35464-F1

Expression profile:

Human Protein Atlas:	ENSG00000092421-SEMA6A

References

1. Z. Anderson, H. Li, T. Riedel, H. Zhu and D. Moshinsky. (2026). Flow Cytometry for Anti-SEMA6A [IPI-mSEM6A.18]. Addgene. https://doi.org/10.57733/addgene.285lqs
2. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-SEMA6A [IPI-mSEM6A.18] in binding assay. Addgene. https://doi.org/10.57733/addgene.l4yumk
3. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-SEMA6A [IPI-mSEM6A.18] in specificity assay. Addgene. https://doi.org/10.57733/addgene.ndwxet

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