Product Datasheet

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Overview

Antigen	CELSR2
Immunogen	Purified recombinant fragment of Mouse CELSR2, corresponding to AA: 1869 - 2372.
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-mCELSR2.52
RRID	AB_3754866
IPI ID	TAB0020111-013
Specificity	CELSR2; Does not cross-react with other CELSRs.
Species	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.

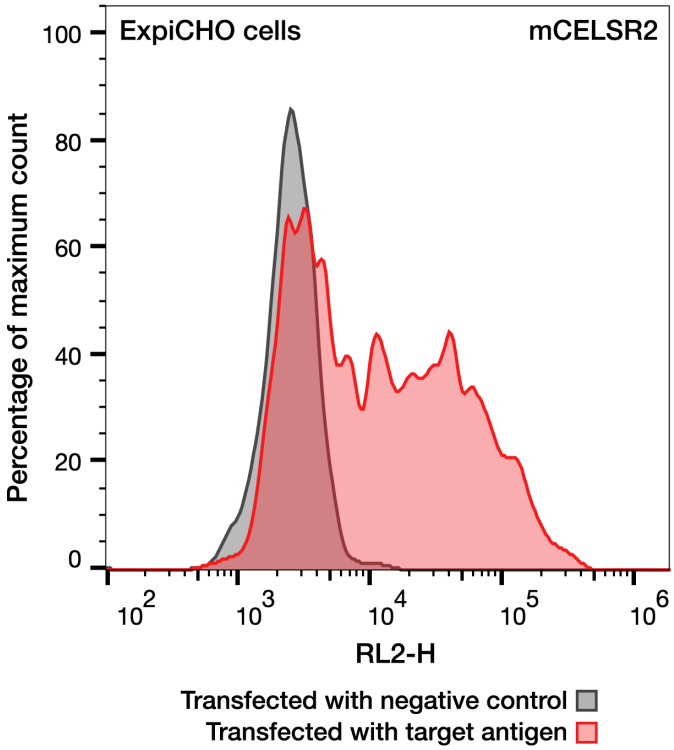
IPI Tested Applications

Applications	Tested Concentration	Result	Reference
Flow Cytometry	0.66-100 µg/mL	Positive	https://doi.org/10.57733/addgene.tly1jz
SPR	4 µg/mL	Positive	https://doi.org/10.57733/addgene.wpbh9m
IF- Binding	10 µg/mL	Positive	https://doi.org/10.57733/addgene.x1s87b
IF - Specificity	10 µg/mL	Positive	https://doi.org/10.57733/addgene.i1dks6
IHC	10 µg/mL	Positive	https://doi.org/10.57733/addgene.aqctu1

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Applications

Cell Display



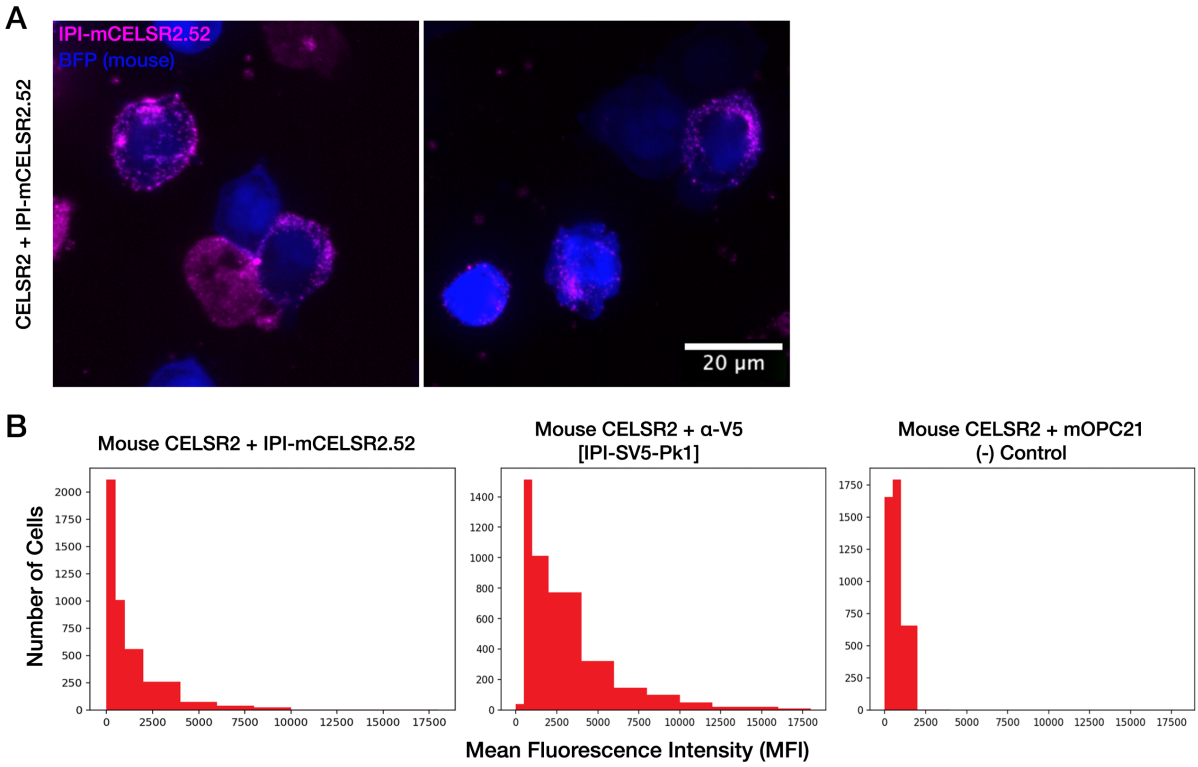
Anti-CELSR2 [IPI-mCELSR2.52] (Addgene # 262535) recognizes Mouse CELSR2.

Histogram from FACS analysis on ExpiCHO cells transfected with mouse CELSR2 (red), or B7H3 negative control (gray). Cells expressing mouse CELSR2 were labeled with Anti-CELSR2 [IPI-mCELSR2.52] and Alexa Fluor 647 F(ab')2 goat anti-rabbit IgG Fc fragment (Jackson ImmunoResearch, 111-606-046). Note that human CELSR2 was not tested. 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.tly1jz

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 mouse CELSR2 showed reactivity towards mouse CELSR2 with observed EC_{50} value of 21.58 nM.

Immunofluorescence (IF) – Binding

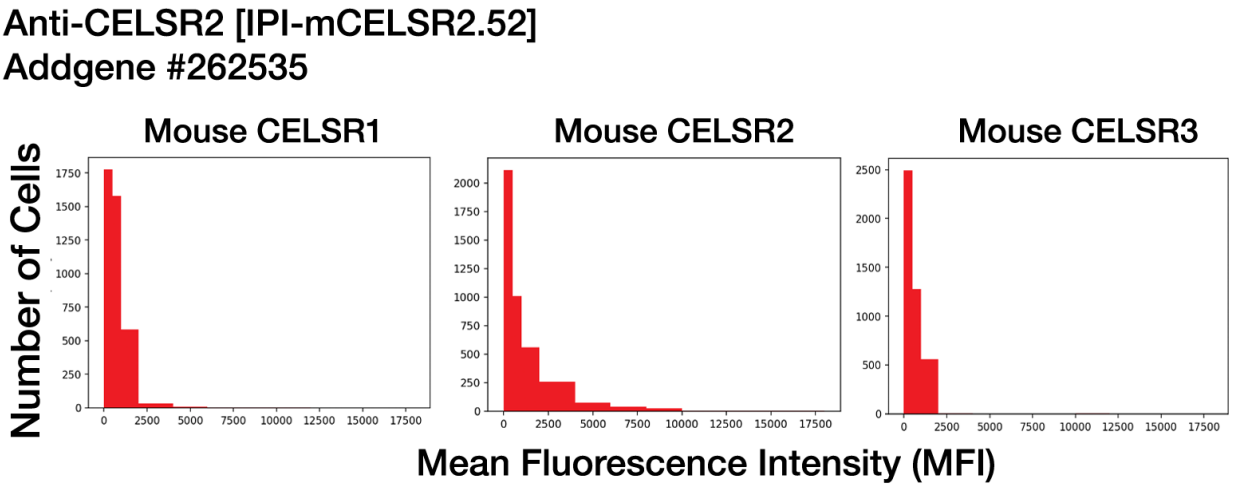


Anti-CELSR2 [IPI-mCELSR2.52] (Addgene# 262535) shows binding to mouse CELSR2.

A) Immunofluorescence (IF) of ExpiCHO cells transfected with mouse (Blue/BFP) CELSR2 and labeled with Anti-CELSR2 [IPI-mCELSR2.52] (Addgene #262535) in magenta. Images taken at 40X. Scale bar = 20 uM. B) Combined quantification of multiple images of the same transfected cells taken at 10X magnification. 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-mCELSR2.52 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 20000. IPI-mCELSR2.52 staining of mouse CELSR2 is shown in first (left) and compared to a positive (middle) and negative (right) control. For both panels, IPI-mCELSR2.52 was used at 10 µg/mL (1:100 dilution).

doi: https://doi.org/10.57733/addgene.x1s87b

Immunofluorescence (IF) - Target Specificity



Family Crossreactivity

	CELSR1		CELSR2		CELSR3	
	Hu	Mo	Hu	Mo	Hu	Mo
IPI-mCELSR2.52	ND		ND	++	ND	

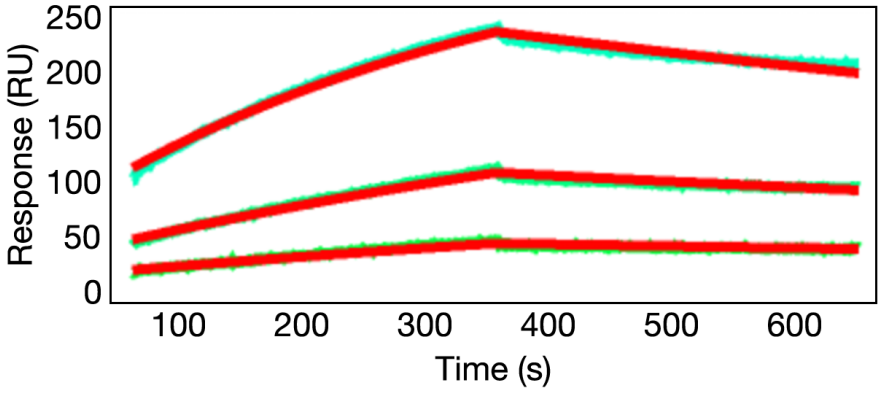
Strong	++
Weak	+
None	

Anti-CELSR2 [IPI-mCELSR2.52] (Addgene# 262535) shows significant binding to mouse CELSR2.

Each graph depicts the combined quantification of multiple images of the same transfected cells taken at 10X magnification. 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-mCELSR2.52 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 20000. IPI-mCELSR2.52 staining of mouse homologs of selected CELSR family members is compared. Binding to human CELSRs was not tested (ND). To test family-wide cross-reactivity, IPI-mCELSR2.52 was used at 10 ug/mL (1:100 dilution).

doi: https://doi.org/10.57733/addgene.i1dks6

Surface Plasmon Resonance (SPR)



Kinetic Parameters

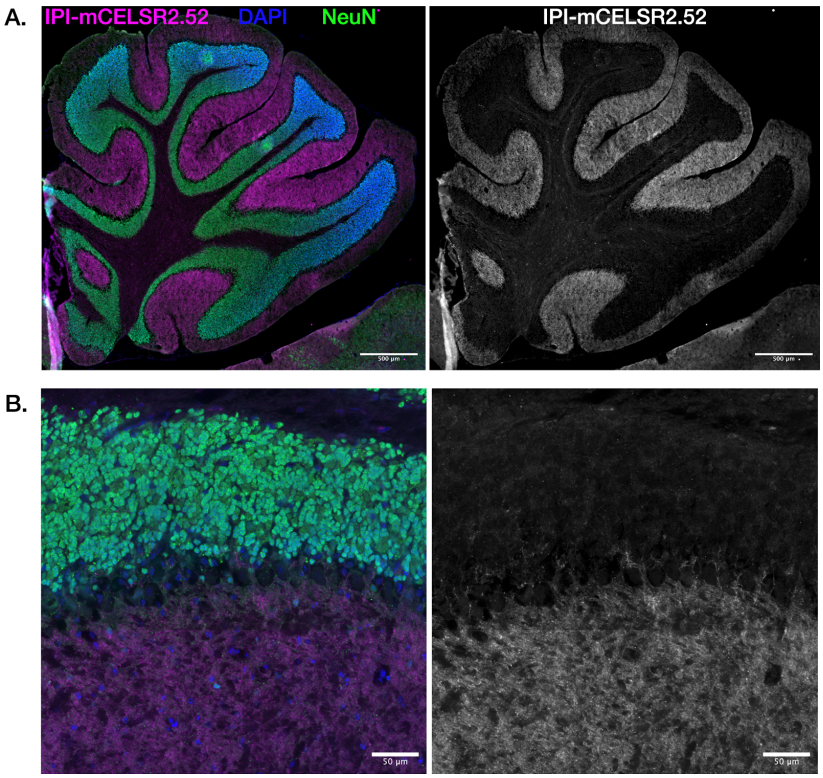
k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	R_{max}	Res SD
4.70×10^{4}	6.30×10^{-4}	1.30×10^{-8}	458.00	2.70

Surface Plasmon Resonance (SPR) kinetics analysis of the interaction between Anti-CELSR2 [IPI-mCELSR2.52] and CELSR2.

SPR binding kinetics were measured on a Carterra LSA-XT (model) using HC30M chips (Carterra, cat. #4279) at 25 °C. Goat anti-rabbit IgG Fc antibody (Jackson ImmunoResearch Laboratories, Cat. #111-005-046) was immobilized via amine coupling, and test antibodies (here IPI-mCELSR2.52) were captured using a 96-channel print-head. Antigens (400 nM to five lower concentrations, 3-fold dilutions) were injected in antigen buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 1 mM CaCl , 1 mM MgCl , 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.

doi: https://doi.org/10.57733/addgene.wpbh9m

Immunohistochemistry (IHC)



Immunohistochemistry (IHC) for Anti-CELSR2 [IPI-mCELSR2.52]

Immunohistochemistry (IHC) of 20 micron cryosections of a Postnatal Day 35 wild type C57BL/6J mouse brain. (A) Images taken at 20x magnification on an Olympus VS200 slide scanner. Scale bar: 500 µm. (B) Images taken at 40x magnification on the ImageXpress confocal HT.ai microscope; magnified region of panel A. Scale bar: 100 µm. Merged images (left) show signal from Anti-CELSR2 [IPI-mCELSR2.52] (Addgene #262535; magenta), DAPI (blue), and anti-NeuN (Millipore MAB377; green), and grayscale images (right) show signal from IPI-mCELSR2.52 only. IPI-mCELSR2.52 was used at 10 µg/mL (1:100 dilution).

doi: https://doi.org/10.57733/addgene.aqctu1

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

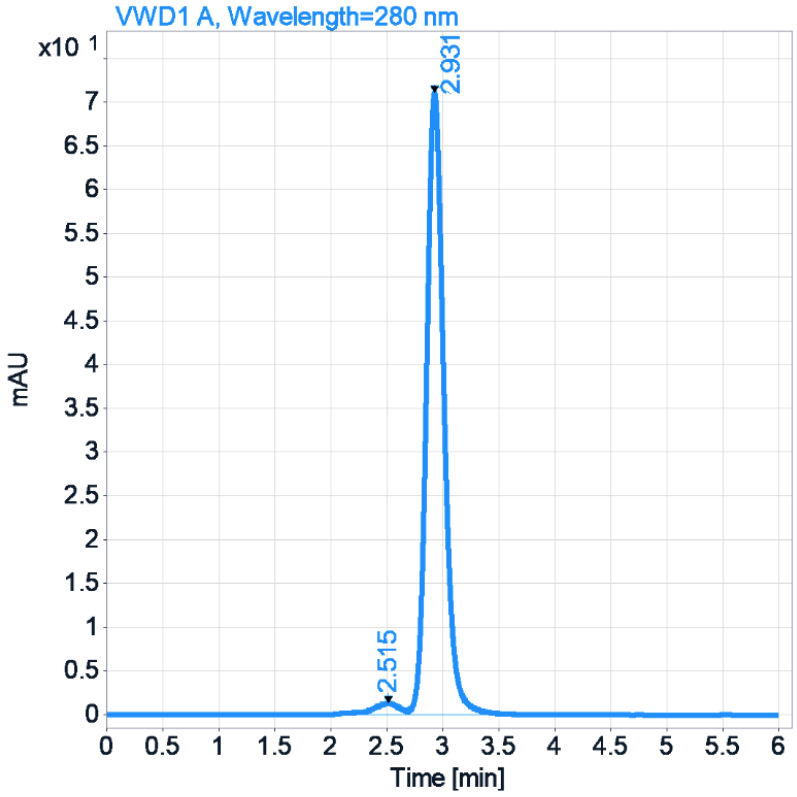
LC Calculated	LC Observed	LC Delta	HC Calculated	HC Observed	HC Delta
22944.51	22944.39	-0.12	49452.83	49458.65	-0.12

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)	Area %	Result
IPI-mCELSR2.52	2.93	97.11	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 CELSR2 (AA: 1869 - 2372):

RIDQPCPRGWWGHPTCGPCNCDVSKGFDPDCNKTSGECHCKENHYRPPGSPTCLLCDCYPTGSLSRVCDPEDGQ
CPCKPGVIGRQCDRCDNPFAEVTTNGCEVNYDSCPRAIEAGIWWPRTRFGLPAAAPCPKGSFGTAVRHCDEHRGW
LPPNLFNCTSVTFSELKGFAERLQRNESGLDSGRSQRLALLLRNATQHTSGYFGSDVKVAYQLATRLLAHESAQRGF
GLSATQDVHFTENLLRVGSALLDAANKRHWELIQQTEGGTAWLLQHYEAYASALAQNMRHTYLSPFTIVTPNIVISVV
RLDKGNFAGTKLPRYEALRGERPPDLETTVILPESVFREMPSMVRSAGPGEAQETEELARRQRRHPELSQGEAVAS
VIIYHTLAGLLPHNYDPDKRSLRVPKRPVINTPVVSISVHDDEELLPRALDKPVTVQFRLLETEERTKPICVFWNHSILV
SGTGGWSARGCEVVFRNESHVSCQCNHMTSFAVLMDMSRRENGEGGGGSGGGGSPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLAQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVNLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLNSTLTVDKSRWQQGNVFSCSVMHEALANHYTQKSLSLSPGKGSGGLNDIFEAQKIEWHEGSGHHHHHH

Sequence information:

HUGO: MGI:1858235
Uniprot: Q9R0M0
Refseq: NM_017392.4

Structural information:

Topology: Multi-pass membrane protein
PDB IDs: -
Alphafold: AF-Q9R0M0-F1

Expression profile:

Human Protein Atlas: ENSG00000143126

References

1. A. Kachare, M. Anuganti, H. Li, M. Sandaire, N. Petrunich, N. Pokhrel Mashock, T. Riedel, and D. Moshinsky. (2026). SPR for Anti-CELSR2 [IPI-mCELSR2.52]. Addgene. https://doi.org/10.57733/addgene.wpbh9m
2. Z. Anderson, H. Li, T. Riedel, H. Zhu and D. Moshinsky. (2026). Flow for Anti-CELSR2 [IPI-mCELSR2.52]. Addgene. https://doi.org/10.57733/addgene.tly1jz
3. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC for Anti-CELSR2 [IPI-mCELSR2.52]. Addgene. https://doi.org/10.57733/addgene.x1s87b
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5. A. Morano, T. Riedel, and D. Moshinsky. (2026). IHC for Anti-CELSR2 [IPI-mCELSR2.52]. Addgene. https://doi.org/10.57733/addgene.aqctu1

How to cite this antibody:

Anti-CELSR2 [IPI-mCELSR2.52] - from Institute for Protein Innovation (IPI) (Addgene #262535; https://n2t.net/addgene:262535; RRID: AB_3754866).

If you publish research with this product, please let us know so that we can cite your paper.