# Product Datasheet

Applications | Antibody Details | Antigen Details | References

## Overview

| | |
|---|---|
| Antigen | CELSR3 |
| Immunogen | Purified recombinant fragment of Mouse CELSR3, corresponding to AA: 2042 - 2529. |
| Host/isotype | Rabbit/IgG |
| Clonality | Recombinant monoclonal |
| Clone name | IPI-mCELSR3.16 |
| RRID | AB_3754867 |
| IPI ID | TAB0019868-013 |
| Specificity | CELSR3; 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. |

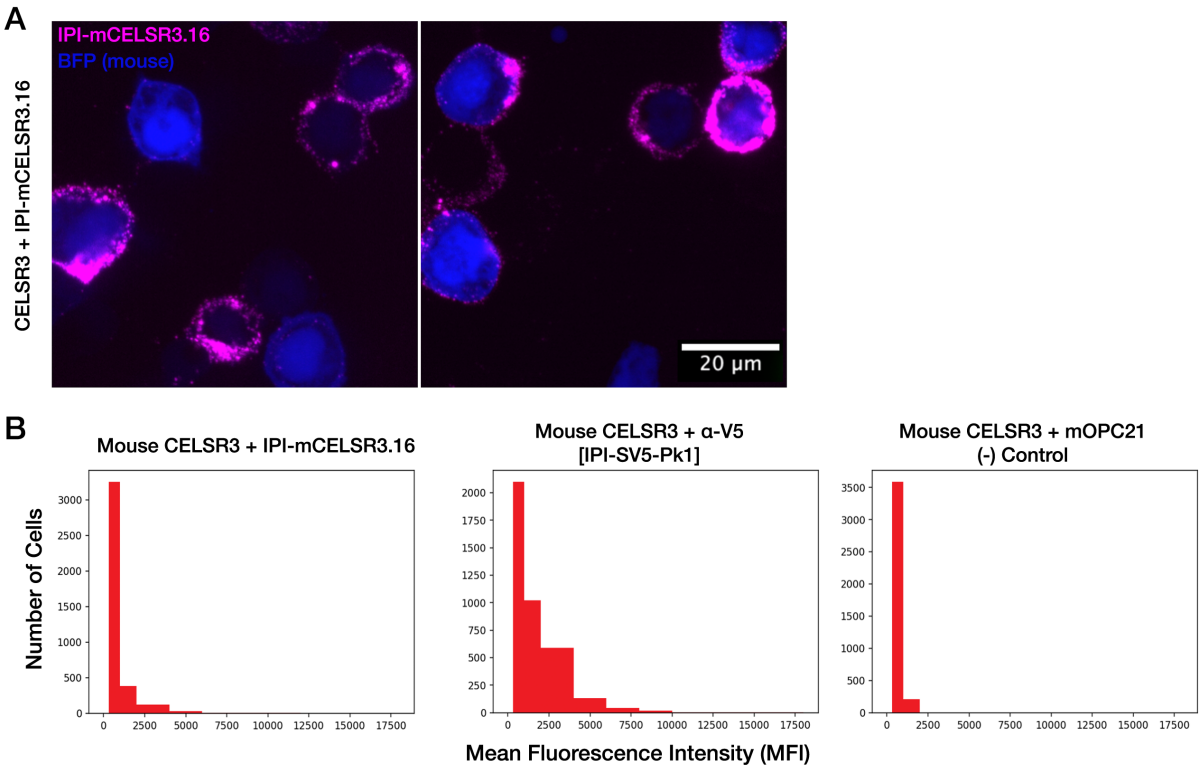
## IPI Tested Applications

| Applications | Tested Concentration | Result | Reference |
|---|---|---|---|
| SPR | 4 µg/mL | Positive | https://doi.org/10.57733/addgene.8cjjwe |
| IF- Binding | 10 µg/mL | Positive | https://doi.org/10.57733/addgene.il8j4y |
| IF - Specificity | 10 µg/mL | Positive | https://doi.org/10.57733/addgene.mmdu7e |
| IHC | 10 µg/mL | Positive | https://doi.org/10.57733/addgene.k8wtyn |

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# Applications

## Immunofluorescence (IF) – Binding

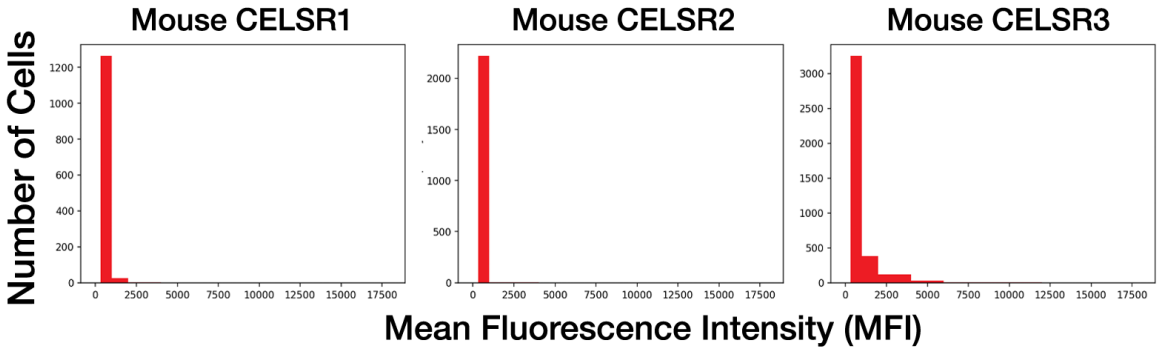



**Anti-CELSR3 [IPI-mCELSR3.16] (Addgene# 262536) shows binding to mouse CELSR3.**

A) Immunofluorescence (IF) of ExpiCHO cells transfected with mouse (Blue/BFP) CELSR3 and labeled with Anti-CELSR3 [IPI-mCELSR3.16] (Addgene #262536) 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-mCELSR3.16 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 20000. IPI-mCELSR3.16 staining of mouse CELSR3 is shown first (left), and compared to a positive (middle) and negative (right) control. For both panels, IPI-mCELSR3.16 was used at 10 µg/mL (1:100 dilution).

doi: https://doi.org/10.57733/addgene.il8j4y

## Immunofluorescence (IF) - Target Specificity




Family Crossreactivity

| | CELSR1 | | CELSR2 | | CELSR3 | |
|---|---|---|---|---|---|---|
| | Hu | Mo | Hu | Mo | Hu | Mo |
| IPI-mCELSR3.16 | ND | | ND | | ND | ++ |

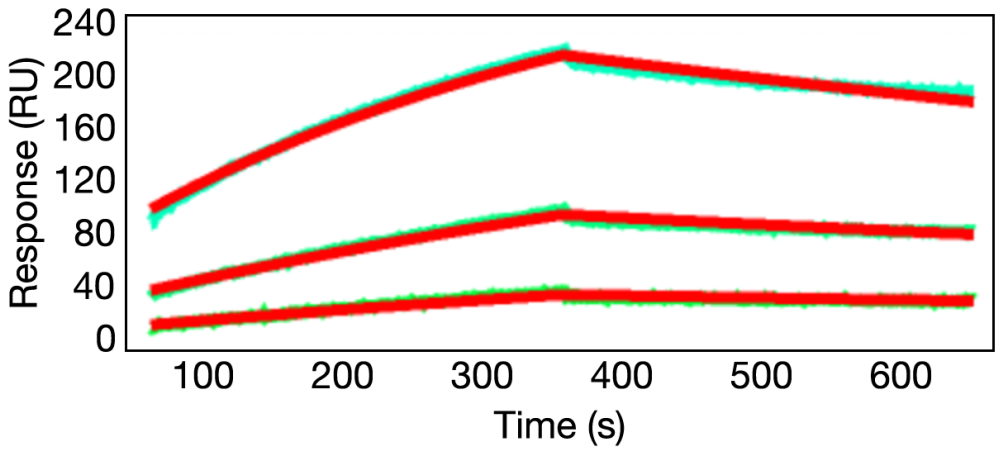
| | |
|---|---|
| Strong | ++ |
| Weak | + |
| None | |

**Anti-CELSR3 [IPI-mCELSR3.16] (Addgene# 262536) shows significant binding to mouse CELSR3.**

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-mCELSR3.16 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 20000. IPI-mCELSR3.16 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-mCELSR3.16 was used at 10 ug/mL (1:100 dilution).

doi: https://doi.org/10.57733/addgene.mmdu7e

## Surface Plasmon Resonance (SPR)




**Kinetic Parameters**

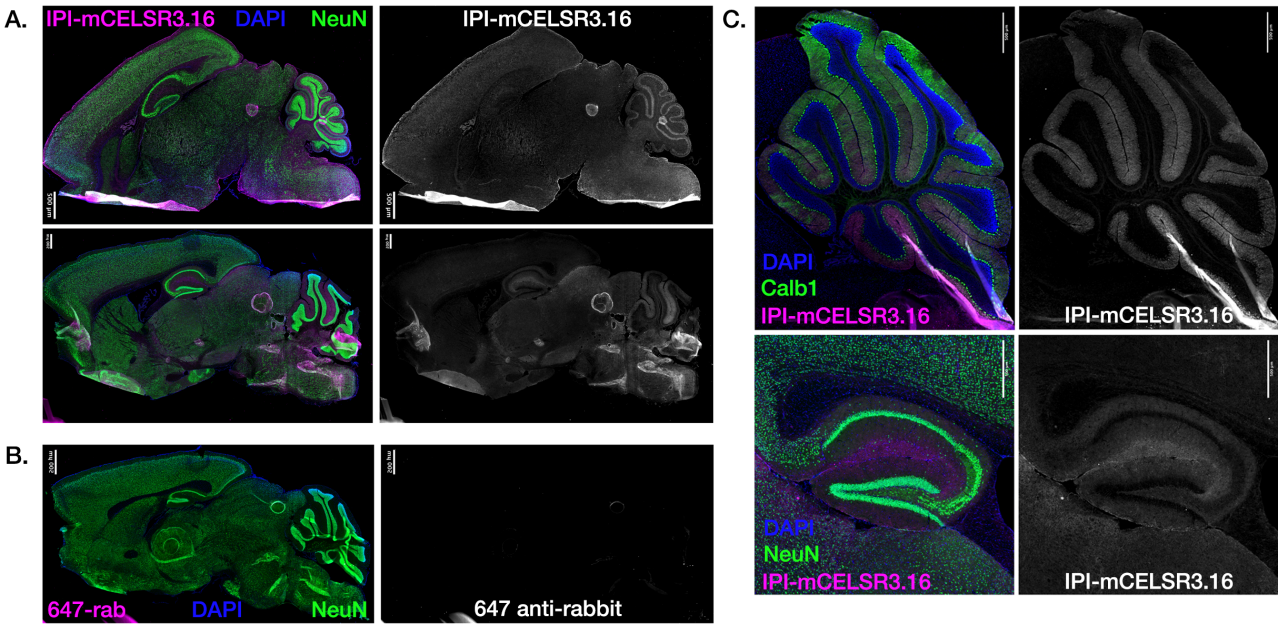
| $k_a$ ($M^{-1}s^{-1}$) | $k_d$ ($s^{-1}$) | $K_D$ (M) | $R_{max}$ | Res SD |
|---|---|---|---|---|
| $1.30\times10^{5}$ | $2.50\times10^{-3}$ | $2.00\times10^{-8}$ | 340.00 | 4.70 |

**Surface Plasmon Resonance (SPR) kinetics analysis of the interaction between Anti-CELSR3 [IPI-mCELSR3.16] and CELSR3.**

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-mCELSR3.16) 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.8cjjwe

## Immunohistochemistry (IHC)




### Immunohistochemistry (IHC) for Anti-CELSR3 [IPI-mCELSR3.16]

Immunohistochemistry (IHC) of 20 micron cryosections of Postnatal Day 10 (top row, A) or Day 35 (bottom row, A; B-C) wild type C57BL/6J mouse brains. Images were taken at 20X magnification on an Olympus VS200 slide scanner. Scale bars = 500 µm. Merged images show signal from IPI-mCELSR3.16 primary (A, C) or no rabbit primary control (B) (magenta), DAPI (blue), and anti-NeuN (A, B, bottom row of C) ( Millipore MAB377) or anti-Calbindin/Calb1 (Millipore SAB4200543), and the grayscale image shows signal from IPI-mCELSR3.16 or no rabbit primary control. Panel A shows whole brain images from P10 and P35 mice stained with IPI-mCELSR3.16. Panel B shows a whole brain control/no primary image from P35 mice. Panel C zooms in on the cerebellum (top) and Hippocampus (bottom) from a P35 adult mouse brain to highlight staining in those regions of the brain. IPI-mCELSR3.16 was used at 10 ug/mL (1:100 dilution).

doi: https://doi.org/10.57733/addgene.k8wtyn

# 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

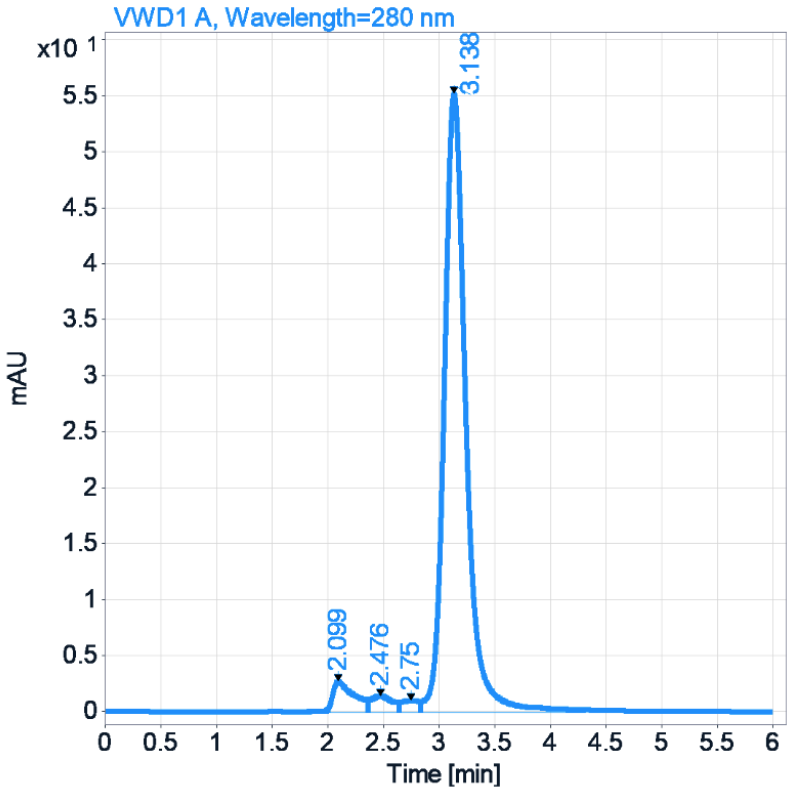
| LC Calculated | LC Observed | LC Delta | HC Calculated | HC Observed | HC Delta |
|---|---|---|---|---|---|
| 22952.44 | 22952.01 | -0.43 | 49913.41 | 49919.67 | 6.26 |

**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-mCELSR3.16 | 3.14 | 91.56 | 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 CELSR3 (AA: 2042 - 2529):**

NKTNGQCHCKEFHYRPRGSDSCLPCDCYPVGSTSRSCAPHSGQCPCRPGALGRQCNSCDSPFAEVTASGCRVLY
DACPKSLRSGVWWPQTKFGVLATVPCPRGALGAAVRLCDEDQGWLEPDLFNCTSPAFRELSLLLDGLELNKTALDT
VEAKKLAQRLREVTGQTDHYFSQDVRVTARLLAYLLAFESHQQGFGLTATQDAHFNENLLWAGSALLAPETGHLWA
ALGQRAPGGSPGSAGLVQHLEEYAATLARNMELTYLNPVGLVTPNIMLSIDRMEHPSSTQGARRYPRYHSNLFRGQ
DAWDPHTHVLLPSQASQPSPSEVLPTSSNAENATASSVVSPPAPLEPESEPGISIVILLVYRALGGLLPAQFQAERRG
ARLPQNPVMNSPVVSVAVFHGRNFLRGVLVSPINLEFRLLQTANRSKAICVQWDPPGPTDQHGMWTARDCELVHR
NGSHARCRCSRTGTFGVLMDASPRERLEGDLELGGGGSGGGGSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLAQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVNLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLNSTLTVDKS
RWQQGNVFSCSVMHEALANHYTQKSLSLSPGKGSGGLNDIFEAQKIEWHEGSGHHHHHH

**Sequence information:**

HUGO: MGI:1858236
Uniprot: Q91ZI0
Refseq: NM_080437.3

**Structural information:**

Topology: Multi-pass membrane protein
PDB IDs: -
Alphafold: AF-Q91ZI0-F1

**Expression profile:**

Human Protein Atlas: ENSG00000008300

## References

1. A. Kachare, M. Anuganti, H. Li, M. Sandaire, N. Petrunich, N. Pokhrel Mashock, T. Riedel, and D. Moshinsky. (2026). SPR for Anti-CELSR3 [IPI-mCELSR3.16]. Addgene. https://doi.org/10.57733/addgene.8cjjwe
2. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC for Anti-CELSR3 [IPI-mCELSR3.16]. Addgene. https://doi.org/10.57733/addgene.il8j4y
3. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC for Anti-CELSR3 [IPI-mCELSR3.16]. Addgene. https://doi.org/10.57733/addgene.mmdu7e
4. A. Morano, T. Riedel, and D. Moshinsky. (2026). IHC for Anti-CELSR3 [IPI-mCELSR3.16]. Addgene. https://doi.org/10.57733/addgene.k8wtyn

**How to cite this antibody:**

Anti-CELSR3 [IPI-mCELSR3.16] - from Institute for Protein Innovation (IPI) (Addgene #262536; https://n2t.net/addgene:262536; RRID: AB_3754867).

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