Product Datasheet

Anti-Neuroligin-2 [IPI-mNLGN2.4]

Applications | Antibody Details | Antigen Details | References

Overview

Antigen	Neuroligin-2
Immunogen	Purified recombinant fragment of Mouse Neuroligin-2, corresponding to AA: 15-678.
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-mNLGN2.4
RRID	AB_3678698
IPI ID	TAB0011256-013-004
Specificity	NLGN2; Does not recognize other NLGNs
Species reactivity	Mouse and human
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.44yyu3
IF – Binding	0.1 µg/mL	Positive	https://doi.org/10.57733/addgene.doarnp
IF – Specificity	0.1 µg/mL	Positive	https://doi.org/10.57733/addgene.bukbq4
SPR	12 µg/mL	Positive	https://doi.org/10.57733/addgene.rkk41b

‡ Not suitable for WB application.

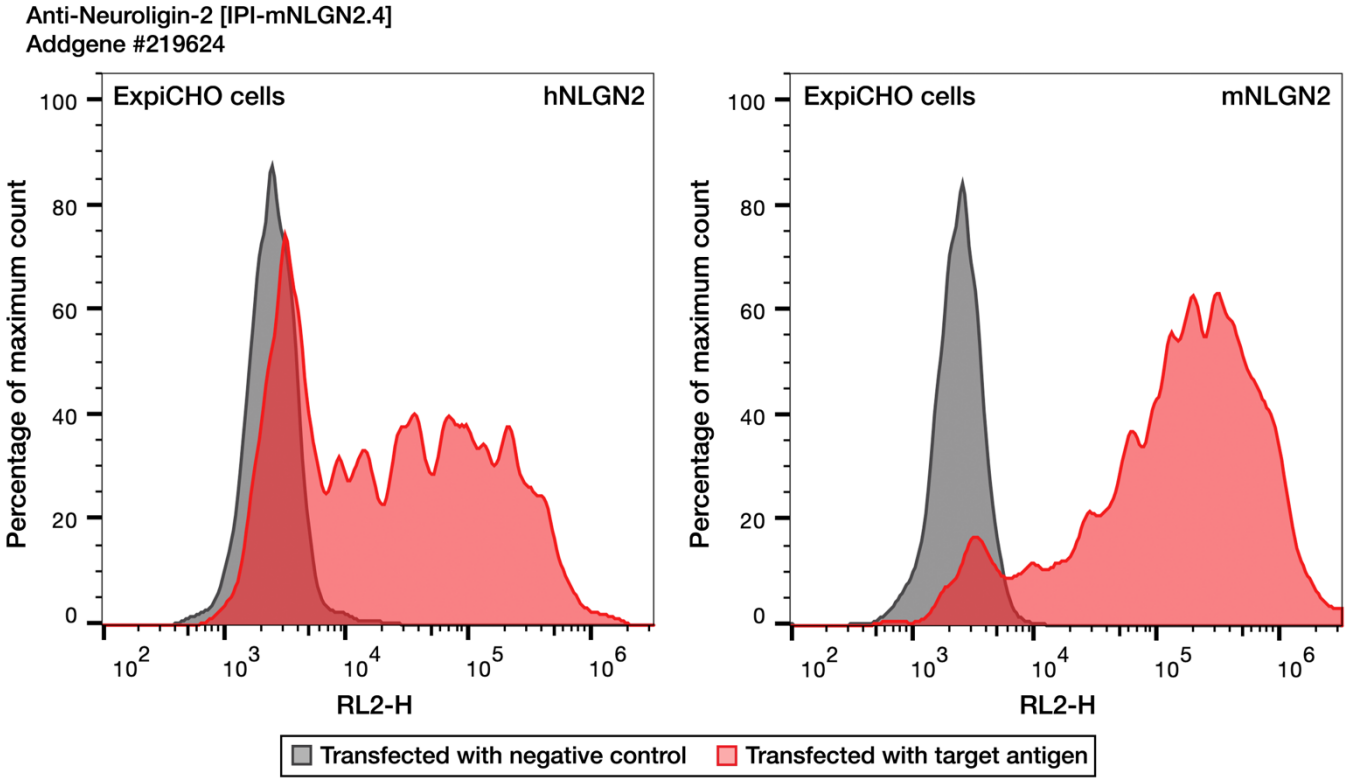
Community Data*

Application	Lab	Reference
IHC	Karl Murray, Ph.D., NeuroMab	https://doi.org/10.57733/addgene.q3i5ja

* Supporting Data is generated by external partner labs, in the process of evaluating IPI antibody panels.

Applications

Flow cytometry

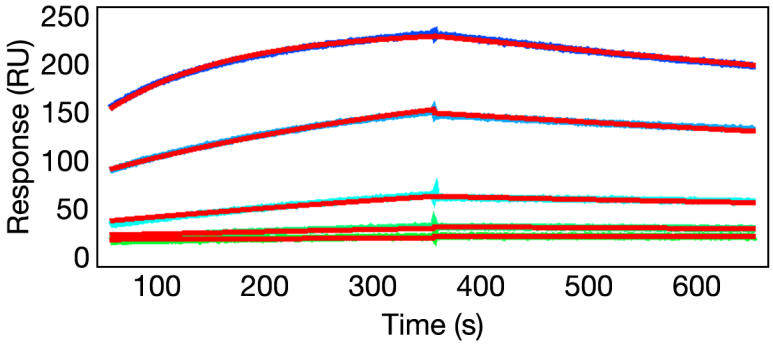


Anti-Neuroligin-2 [IPI-mNLGN2.4] (Addgene #219624) recognizes human and mouse NLGN2. Histogram from FACS analysis on ExpiCHO cells transfected with human or mouse NLGN2 (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) NRXN1b were labeled with Anti-Neuroligin-2 [IPI-mNLGN2.4] 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.44yyu3

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 Neuroligin-2 showed reactivity towards Mouse and human Neuroligin-2 with observed EC50 values of 2.18 nM and 1.69 nM for human and mouse Neuroligin-2 , respectively.

Surface Plasmon Resonance (SPR)

Anti-Neuroligin-2 [IPI-mNLGN2.4]
Addgene #219624

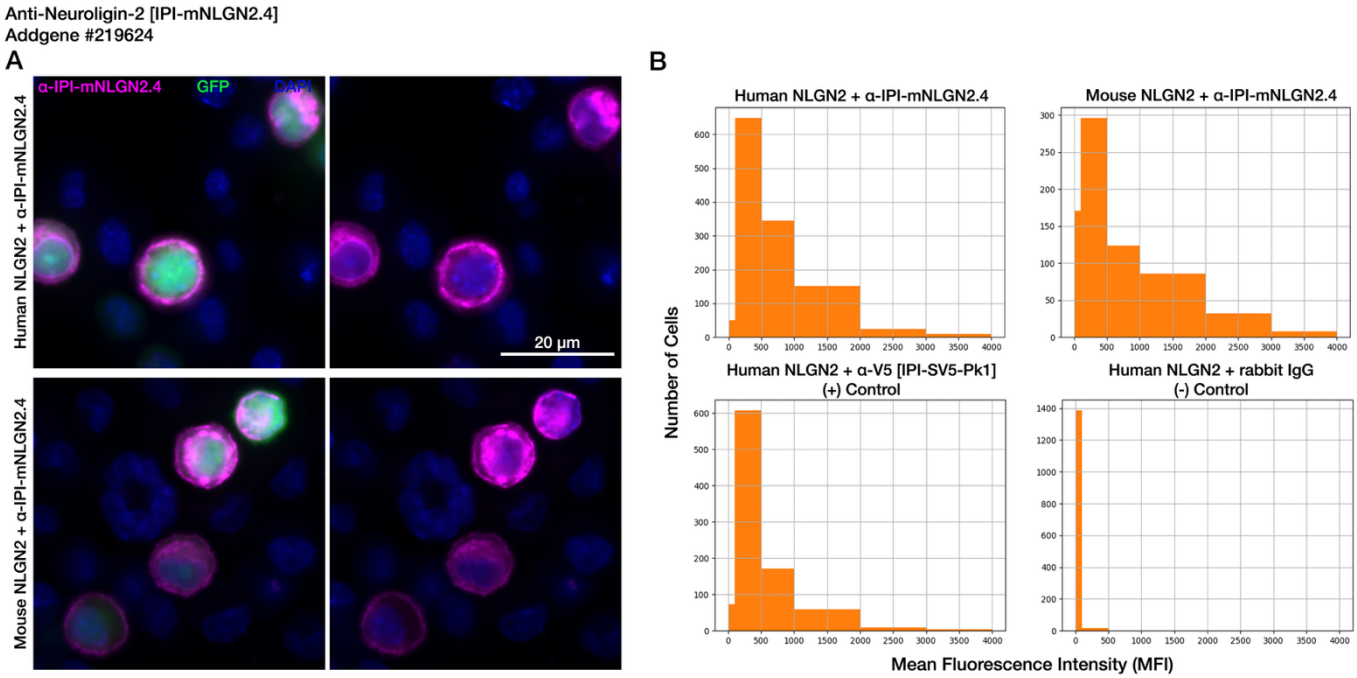


Kinetic Parameters

k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
$4.16x10^{4}$	$5.18x10^{-4}$	$1.25x10^{-8}$

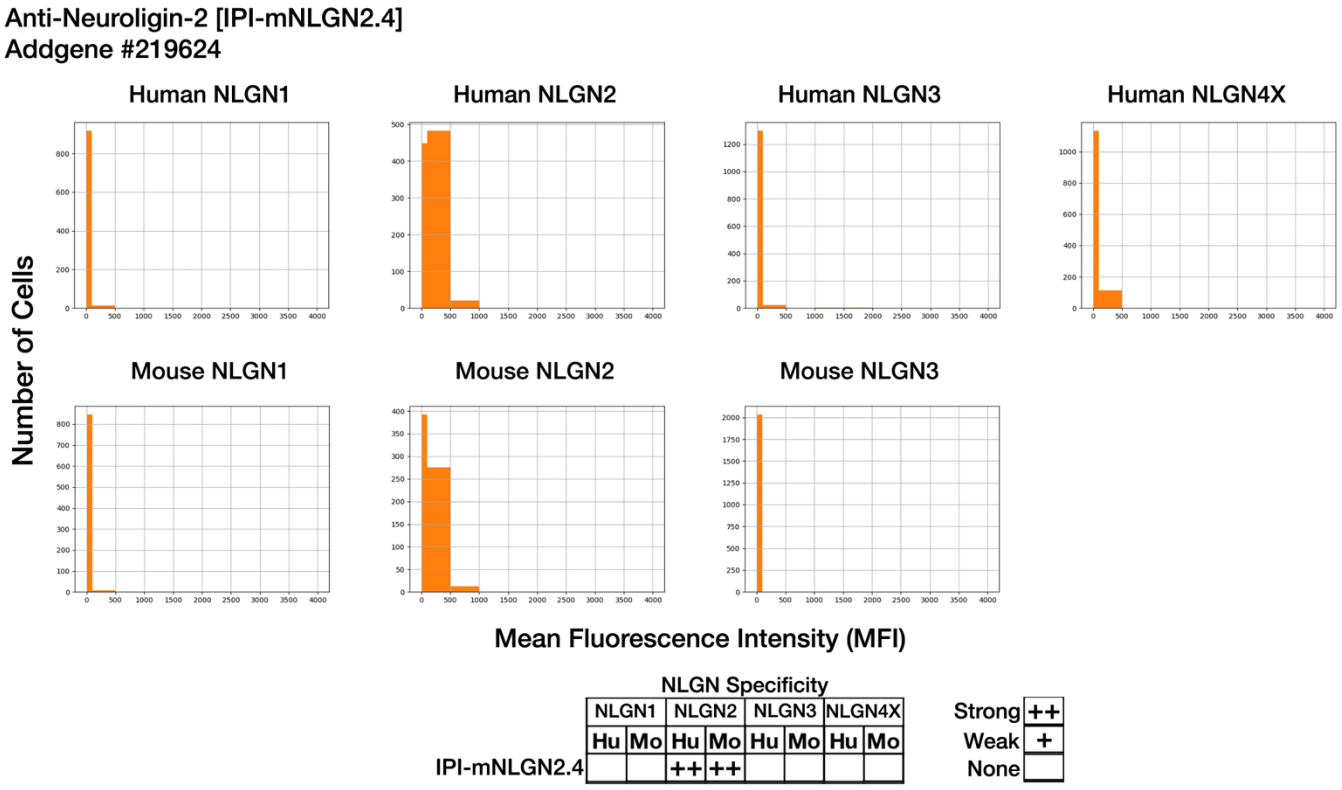
Surface Plasmon Resonance (SPR) kinetics analysis of the interaction between Anti-Neuroligin-2 [IPI-mNLGN2.4] and M. musculus (mouse) NLGN2. SPR binding kinetics were measured on a Carterra LSA using HC30M chips (Carterra, cat. #4279) at 25 °C. Goat anti-rabbit IgG Fc (Jackson ImmunoResearch, cat. #111-005-046) was immobilized via amine coupling, and test antibodies were captured using a 96-channel print-head. Antigens (400 nM to five lower concentrations, 2-fold dilutions) were injected in antigen buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 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, and replotted in OriginPro 2023b. Results show a high-affinity and specific binding event between the antibody and antigen. doi: https://doi.org/10.57733/addgene.rkk41b

Immunofluorescence (IF) – Species Reactivity



Anti-Neuroligin-2 [IPI-mNLGN2.4] (Addgene#219624) shows binding to human and mouse NLGN2. A) Immunofluorescence (IF) of ExpiCHO cells transfected with human and mouse NLGN2. Widefield images taken at 40X magnification on an EV0S-m7000 microscope and deconvolved using the Richardson-Lucy algorithm in the FIJI DeconvolutionLab 2 plugin. Left image shows all 3 channels (IPI-mNLGN2.4, GFP (transfection control) and DAPI) while the right image only shows IPI-mNLGN2.4 and DAPI. B) Combined quantification of multiple images of the same transfected cells taken at 10X magnification. GFP-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-mNLGN2.4 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 4095 (saturation). IPI-mNLGN2.4 staining of human NLGN2 is shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-mNLGN2.4 was used at 0.1 ug/mL (1:10,000 dilution). doi: https://doi.org/10.57733/addgene.doarnp

Immunofluorescence (IF) – Target Specificity



NLGN Specificity

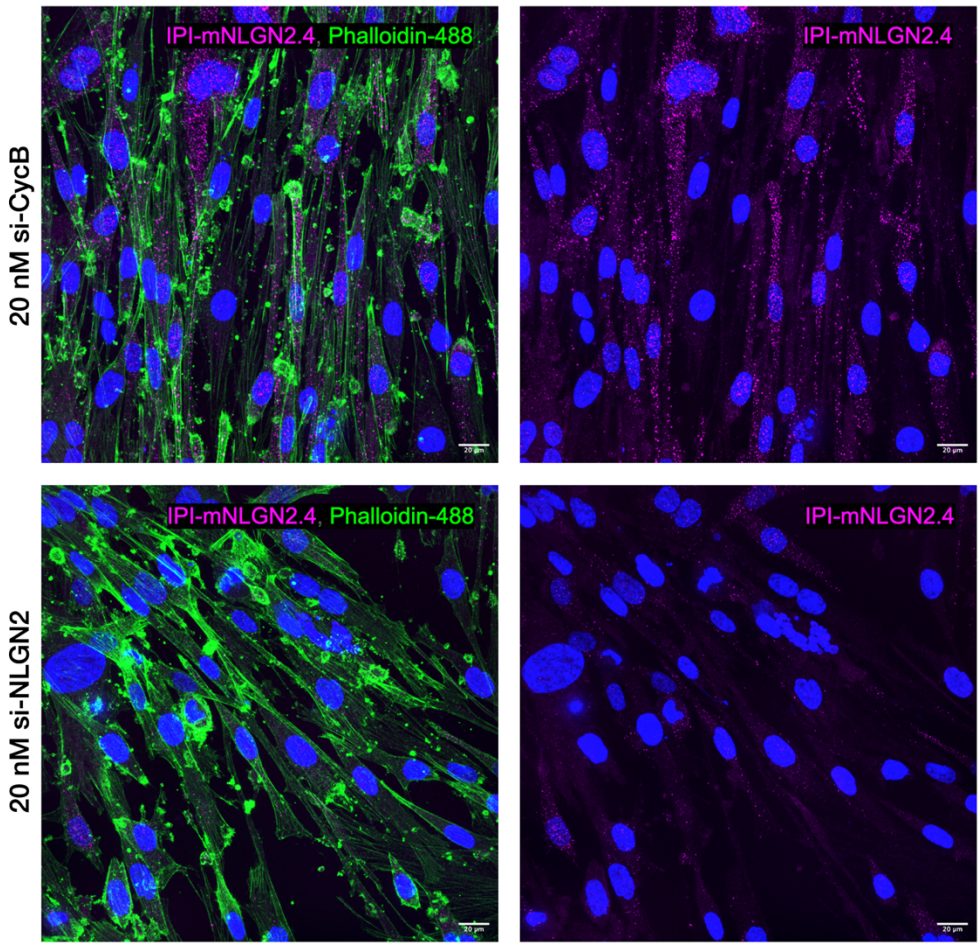
	NLGN1		NLGN2		NLGN3		NLGN4X	
	Hu	Mo	Hu	Mo	Hu	Mo	Hu	Mo
IPI-mNLGN2.4			++	++				

Strong	++
Weak	+
None	

Anti-Neuroligin-2 [IPI-mNLGN2.4] (Addgene#219624) shows specific binding to human and mouse NLGN2. Each graph depicts the combined quantification of multiple images of the same transfected cells taken at 10X magnification. GFP-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-mNLGN2.4 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 4095 (saturation). IPI-mNLGN2.4 staining of human and mouse variants of each NRXNa family member is compared on the top and bottom rows. To test family-wide cross-reactivity, IPI-mNLGN2.4 was used at 0.1 ug/mL (1:10,000 dilution). doi: https://doi.org/10.57733/addgene.bukbq4

Immunofluorescence (IF) – Endogenous

Anti-Neuroligin-2 [IPI-mNLGN2.4]
Addgene #219624



Anti-Neuroligin-2 [IPI-mNLGN2.4] (Addgene#219624) shows binding only to cells expressing NLGN2. Immunofluorescence (IF) of U118-MG cells treated with 20 nM siRNA against housekeeping gene cyclophilin B (top row) and 20 nM siRNA against NLGN2.4 for 90 hours, then stained for NLGN2 expression using IPI-mNLGN2.4 and for actin with Alexa Fluor 488-conjugated phalloidin. Confocal images taken at 40X magnification on an ImageXpress HT confocal.ai microscope. Left images show all 3 channels (IPI-mNLGN2.4, actin, and DAPI) while the right images only show IPI-mNLGN2.4 and DAPI. For both panels, IPI-mNLGN2.4 was used at 5 ug/mL (1:200 dilution). doi: https://doi.org/10.57733/addgene.dwifpq

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

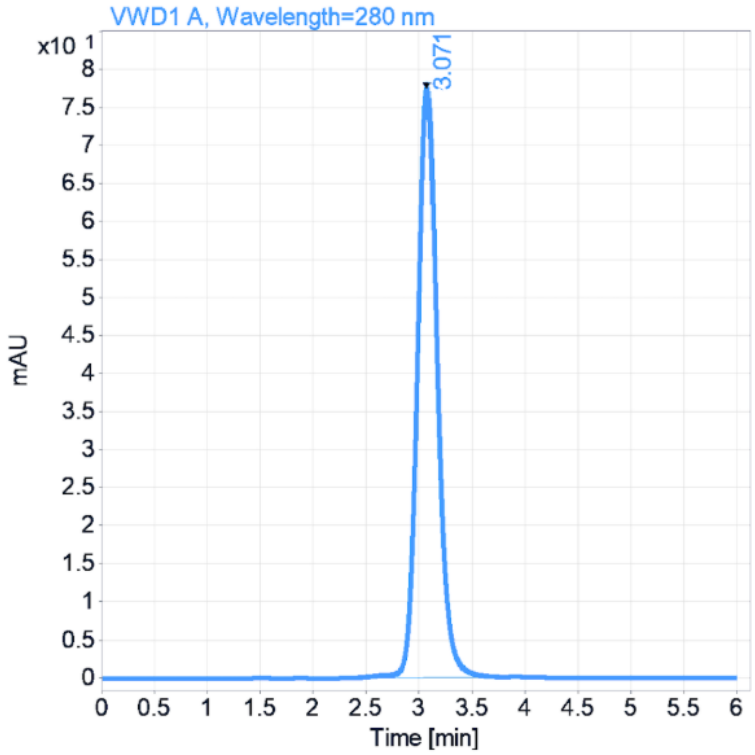
LC-MS: Intact mass analysis via LC-MS methods allows for confirmation antibody mass, and to identify any product-related variants such as glycosylation. Before conducting intact mass analysis via LC-MS, the antibody was reduced to its heavy chain (HC) and light chain (LC). This process allows for confirmation 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-mNLGN2.4	49951.84	49955.81	3.97	22618.93	22619.05	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 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): SEC is a protein purification technique that separates molecules based on size.



	RT (min)	Width (min)	Area	Height	Area %	Result
IPI-mNLGN2.4	3.071	0.1965	990.7004	77.31	100	Pass

Antigen Details

Immunogen design:

cDNA of Mouse Neuroligin-2 with C-terminal His-, Avi-, and HA-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:

>Mouse NLGN2 (AA: 15-678)
DYKDDDDKGSGQRGGGGPGGGAPGGPGLGLGSLGEERFPVVNTAYGRVRGVRRELNNEILGPVVQFL
GVPYATPPLGARRFQPPEAPASWPGVRNATTLPPACPQNLHGALPAIMLPVWFTDNLEAAATYVQNQS
EDCLYLNLYVPTEDGPLTKKRDEATLNPPDTDIRDSGKKPVMLFLHGGSYMEGTGNMFDGSVLAAYGN
VIVVTLNYRLGVLGFLSTGDQAAKGNYGLLDQIQALRWLSENIAHFGGDPERITIFGSGAGASCVNLLILSH
HSEGLFQKAIAQSGTAISSWSVNYQPLKYTRLLAAKVGCDREDSTEAVECLRRKSSRELVDQDVQPARY
HIAFGPVVDGDVVPDDPEILMQQGEFLNYDMLIGVNQGEGLKFVEDSAESEDGVSASAFDFTVSNFVDNL
YGYPEGKDVLRETIKFMYTDWADRDNGEMRRKTLLALFTDHQWVAPAVATAKLHADYQSPVYFYTFYH
HCQAEGRPEWADAAHGDELPYVFGVPMVGATDLFPCNFSKNDVMLSAVVMTYWTNFAKTGDPNQPVP
QDTKFIHTKPNRFEEVVWSKFNSKEKQYLHIGLKPRVRDNYRANKVAFWLELVPHLHNLHTELFTTTTRL
PPYATRWPPRTPGPGTSGTRRPPPPATLPPESDIDLGPRAYDRFPGDSRDYSTELSGSGGLNDIFEAQKI
EWHEGSGHHHHHHHH

Sequence information:

HUGO:	MGI:2681835
Uniprot:	Q69ZK9
Refseq:	NM_001364137.1

Structural information:

Topology:	Single-pass type I membrane protein
PDB IDs:	5XEQ;8GS4
Alphafold:	AF-Q69ZK9-F1

Expression profiles:

Human Protein Atlas: ENSMUSG00000051790

References

1. Z. Anderson, H. Li, T. Riedel, H. Zhu and D. Moshinsky. (2026). Flow Cytometry for Anti-Neuroligin-2 [IPI-mNLGN2.4]. Addgene. https://doi.org/10.57733/addgene.44yyu3
2. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-Neuroligin-2 [IPI-mNLGN2.4] in binding assay. Addgene. https://doi.org/10.57733/addgene.doarnp
3. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-Neuroligin-2 [IPI-mNLGN2.4] in specificity assay. Addgene. https://doi.org/10.57733/addgene.bukbq4
4. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-Neuroligin-2 [IPI-mNLGN2.4] in siRNA KD assay. Addgene. https://doi.org/10.57733/addgene.dwifpq
5. A. Kachare, M. Anuganti, T. Riedel, and D. Moshinsky. (2026). Anti-Neuroligin-2 [IPI-mNLGN2.4] in Surface Plasmon Resonance (SPR). Addgene. https://doi.org/10.57733/addgene.rkk41b
6. K. Murray. (2026). IHC for IPI-mNLGN2.4. Addgene. https://doi.org/10.57733/addgene.q3i5ja

How to cite this antibody:
Anti-Neuroligin-2 [IPI-mNLGN2.4] - from Institute for Protein Innovation (IPI) (Addgene #219624; http://n2t.net/addgene:219624; RRID: AB_3678698).

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