

Product Datasheet

[Anti-Neuroigin-3 \[IPI-mNLGN3.3\]](#)

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Overview

Antigen	Neuroigin-3
Immunogen	Purified recombinant fragment of Mouse Neuroigin-3, corresponding to AA: 36-684.
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-mNLGN3.3
RRID	AB_3678699
IPI ID	TAB0011271-013-004
Specificity	NLGN3; 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.jnwhsv
IF – Binding	1 µg/mL	Positive	https://doi.org/10.57733/addgene.eeh9jx
IF – Specificity	1 µg/mL	Positive	https://doi.org/10.57733/addgene.yv9ng1
SPR	3 µg/mL	Positive	https://doi.org/10.57733/addgene.ard2d9

[†] Not suitable for WB application.

Community Data*

Application	Lab	Reference
IHC	Karl Murray, Ph.D., NeuroMab	https://doi.org/10.57733/addgene.qzec1p
IHC-KO	Peter Scheiffele, Ph.D., University of Basel	https://doi.org/10.57733/addgene.vac25e

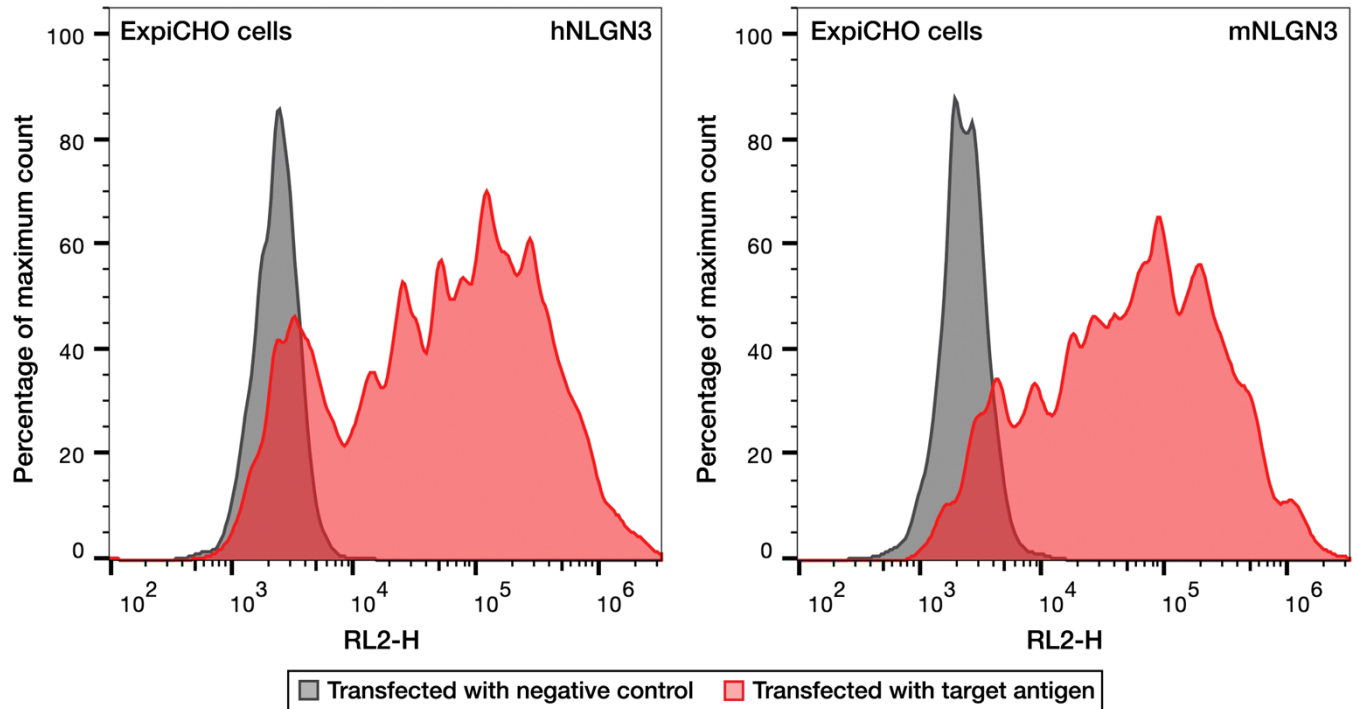
* Supporting Data is generated by external partner labs, in the process of evaluating IPI antibody panels.

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Applications

Flow cytometry

Anti-Neurologin-3 [IPI-mNLGN3.3]
Addgene #219625



Anti-Neurologin-3 [IPI-mNLGN3.3] (Addgene #219625) recognizes human and mouse NLGN3.

Histogram from FACS analysis on ExpiCHO cells transfected with human or mouse NLGN3 (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) NRXN1b were labeled with Anti-Neurologin-3 [IPI-mNLGN3.3] 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.jnwshv>

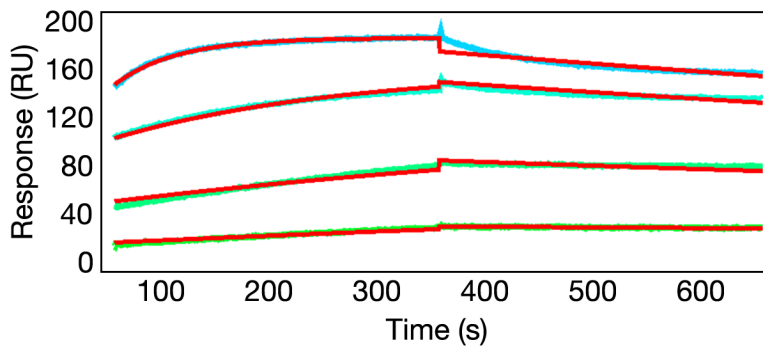
EC₅₀ (data not shown): A fourteen-point titration of antibody concentrations, ranging from 660 nM (0.1 mg/mL) to 4.42 pM with a 1:2.5 dilution factor, against human and mouse Neurologin-3 showed reactivity towards Mouse and human Neurologin-3 with observed EC₅₀ values of 0.19 nM and 0.13 nM for human and mouse Neurologin-3, respectively.

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Surface Plasmon Resonance (SPR)

Anti-Neuroigin-3 [IPI-mNLGN3.3]

Addgene #219625



Kinetic Parameters

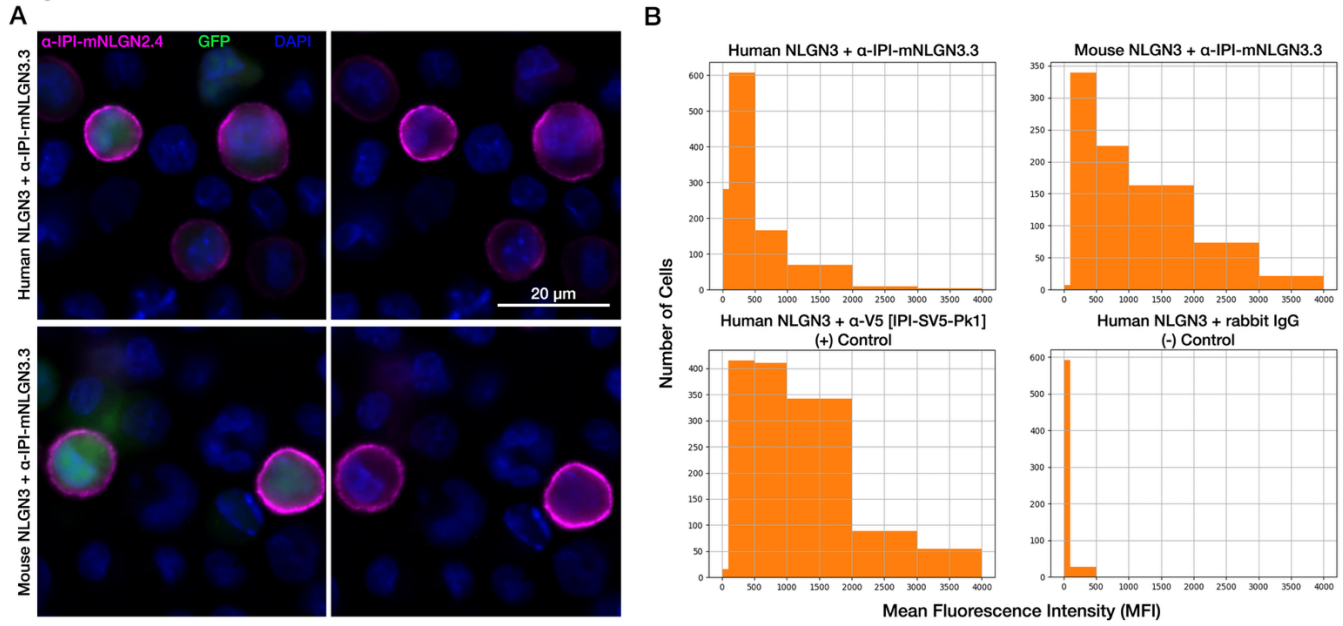
k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
3.38×10^5	4.58×10^{-4}	1.35×10^{-9}

Surface Plasmon Resonance (SPR) kinetics analysis of the interaction between Anti-Neuroigin-3 [IPI-mNLGN3.3] and *M. musculus* (mouse) NLGN3. 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 pH7.4, 150 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂, 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 k_a , k_d , and K_D using Carterra Kinetics software v1.9.2.44.63, and replotted in OriginPro2023b. Results show a high-affinity and specific binding event between the antibody and antigen. doi: <https://doi.org/10.57733/addgene.ard2d9>

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Immunofluorescence (IF) – Species Reactivity

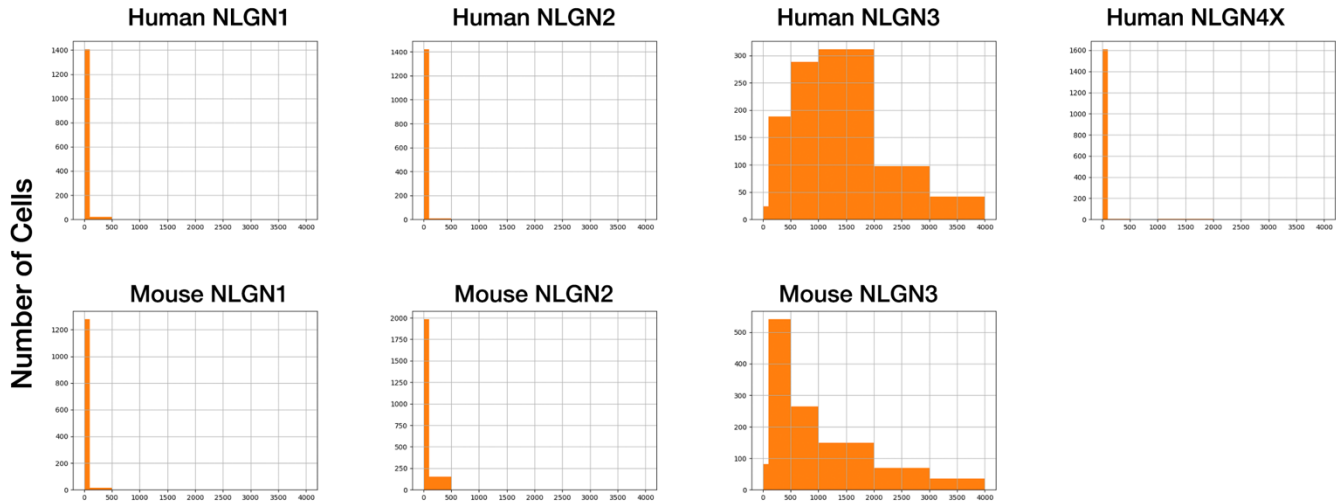
Anti-Neuroigin-3 [IPI-mNLGN3.3]
Addgene #219625



Anti-Neuroigin-3 [IPI-mNLGN3.3] (Addgene#219625) shows binding to human and mouse NLGN3. A) Immunofluorescence (IF) of ExpiCHO cells transfected with human (top) and mouse (bottom) NLGN3. Widefield images taken at 40X magnification on an EVOS-m7000 microscope and deconvolved using the richardson-lucy algorithm in the FIJI deconlab plugin. Left images show all 3 channels (IPI-mNLGN3.3, GFP (transfection control) and DAPI) while the right images only show IPI-mNLGN3.3 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-mNLGN3.3 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 4095 (saturation). mNLGN3.3 staining of human (left) and mouse (right) NLGN3 is shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-mNLGN3.3 was used at 1 ug/mL (1:1000 dilution). doi: <https://doi.org/10.57733/addgene.eeh9jx>

Immunofluorescence (IF) – Target Specificity

Anti-Neuroigin-3 [IPI-mNLGN3.3]
Addgene #219625



Mean Fluorescence Intensity (MFI)

		NLGN Specificity									
		NLGN1		NLGN2		NLGN3		NLGN4X			
		Hu	Mo	Hu	Mo	Hu	Mo	Hu	Mo	Strong	++
IPI-mNLGN3.3						++	++			Weak	+
										None	

Anti-Neuroigin-3 [IPI-mNLGN3.3] (Addgene#219625) shows binding to human and mouse NLGN3 only. 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-mNLGN3.3 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 4095 (saturation). IPI-NLGN3.3 staining of human and mouse variants of each NLGN family member is compared on the top and bottom rows. To test family-wide cross-reactivity, IPI-NLGN3.3 was used at 1 ug/mL (1:1,000 dilution). doi: <https://doi.org/10.57733/addgene.yv9ng1>

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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.

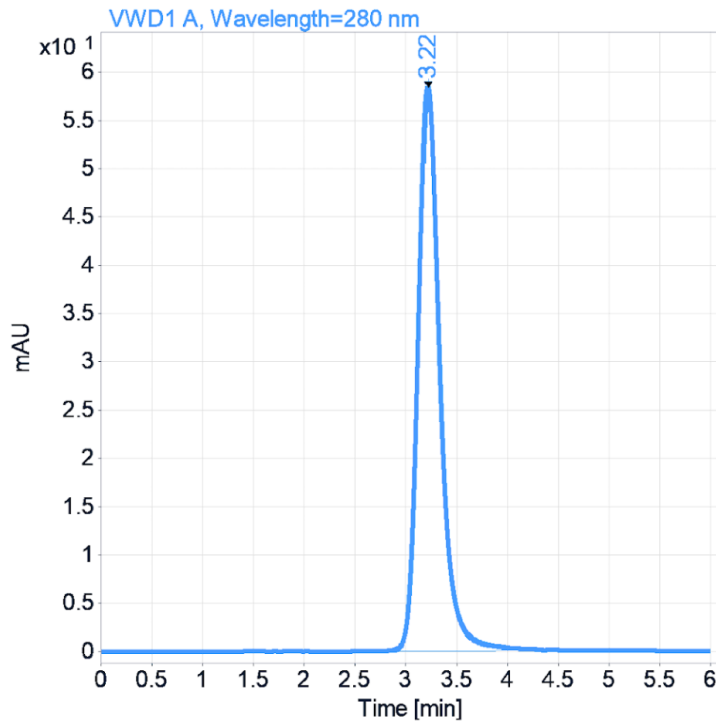
	HC MW (Da) <i>Calculated</i>	HC MW (Da) <i>Observed</i>	HC MW (Da) <i>Delta</i>	LC MW (Da) <i>Calculated</i>	LC MW (Da) <i>Observed</i>	LC MW (Da) <i>Delta</i>
IPI-mNLGN3.3	50615.71	50621.56	5.84	22944.46	22944.75	0.29

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.

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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-mNLGN3.3	3.22	0.223	859.2625	58.915	100	Pass

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Antigen Details

Immunogen design:

cDNA of Mouse Neurologin-3 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 NLGN3 (AA: 36-684)

DYKDDDDKGGSGAPAPTVNTHFGKLRGARVPLPSEILGPVDQYLGVPYAAPPIGEKRFLPPEPPPSWSGIR
NATHFPPVCPQNIHTAVPEVMLPVWFTANLDIVATYIQEPNEDCLYLNYYVPTEDGSGAKKQGEDLADND
GDEDEDIRDSGAKPVMVYIHGGSYMEGTGNMIDGSVLASYGNVIVITLNYRVGVLGFLSTGDQAAKGN
GLLDQIQALRWVSENIFFGGDPRRITVFGSGIGASCVSLLTSLHHSEGLFQRAIIQSGSALSSWAVNYQP
VKYTSLLADKVGCVLDTVDMVDCLRQKSAKELVEQDIQPARYHVAFGPVIDGDVIPDDPEILMEQGEFL
NYDIMLGVNQGEGLKFVEGVVDPEDGVSGTDFDYSVSNFVDNLYGYPEGKDTLRETIFMYTDWADRD
NPETRRTLVALFTDHQWVEPSVVTADLHARYGSPTYFYAFYHHCQSLMKPAWSDAAHGDEVYVFGV
PMVGPTDLFPCNFSKNDVMLSAVVMTYWTNFAKTGDPNKPVPQDTKFIHTKANRFEEVAWSKYNPRDQ
LYLHIGLKPRVRDHYRATKVAFWKHLVPHLYNLHDMFHYTSTTTKVPPDTHSSHITRRPNGKTWSTKR
PAISPAYSNEENAPGSWNGDQDAGPLLLENPRDYSTEGSGGLNDIFEAQKIEWHEGSGHHHHHHHHH

Sequence information:

HUGO: MGI:2444609
Uniprot: Q8BYM5
Refseq: NM_172932.4

Structural information:

Topology: Single-pass type I membrane protein
PDB IDs: 8GS3
AlphaFold: AF-Q8BYM5-F1

Expression profiles:

Human Protein Atlas: ENSG00000196338

References

1. Z. Anderson, H. Li, T. Riedel, H. Zhu and D. Moshinsky. (2026). Flow Cytometry for Anti-Neuroigin-3 [IPI-mNLGN3.3]. Addgene. <https://doi.org/10.57733/addgene.jnwHSV>
2. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-Neuroigin-3 [IPI-mNLGN3.3] in binding assay. Addgene. <https://doi.org/10.57733/addgene.eeh9jx>
3. A. Morano, T. Riedel, and D. Moshinsky. (2026). ICC/IF for Anti-Neuroigin-3 [IPI-mNLGN3.3] in specificity assay. Addgene. <https://doi.org/10.57733/addgene.yv9ng1>
4. A. Kachare, M. Anuganti, T. Riedel, and D. Moshinsky. (2026). Anti-Neuroigin-3 [IPI-mNLGN3.3] in Surface Plasmon Resonance (SPR). Addgene. <https://doi.org/10.57733/addgene.ard2d9>
5. K. Murray. (2026). IHC for IPI-mNLGN3.3. Addgene. <https://doi.org/10.57733/addgene.qzec1p>
6. P. Scheiffele. (2026). IHC for IPI-mNLGN3.3. Addgene. <https://doi.org/10.57733/addgene.vac25e>

How to cite this antibody:

Anti-Neuroigin-3 [IPI-mNLGN3.3] - from Institute for Protein Innovation (IPI) (Addgene #219625; <http://n2t.net/addgene:219625>; RRID: AB_3678699).

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