Product Datasheet

Anti-Neurexin-1-beta [IPI-NRXN1b.35]

Applications | Antibody Details | Antigen Details | References

Overview

Antigen	Neurexin-1-beta
Immunogen	Purified recombinant fragment of Mouse Neurexin-1-beta, corresponding to AA: 50-397.
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-NRXN1b.35
RRID	AB_3678704
IPI ID	TAB0013517-013-002
Specificity	NRXN1b; Cross-reacts with NRXNa (weak) and NRXN3b
Species reactivity	Human and mouse
Amount	100 µg
Concentration	1 mg/mL
Purification	Expressed in HEK293 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.rmgf43
IF – Binding	1 µg/mL	Positive	https://doi.org/10.57733/addgene.y6p77c
IF – Specificity	1 µg/mL	Positive	https://doi.org/10.57733/addgene.4dfaa2

‡ Not suitable for WB application.

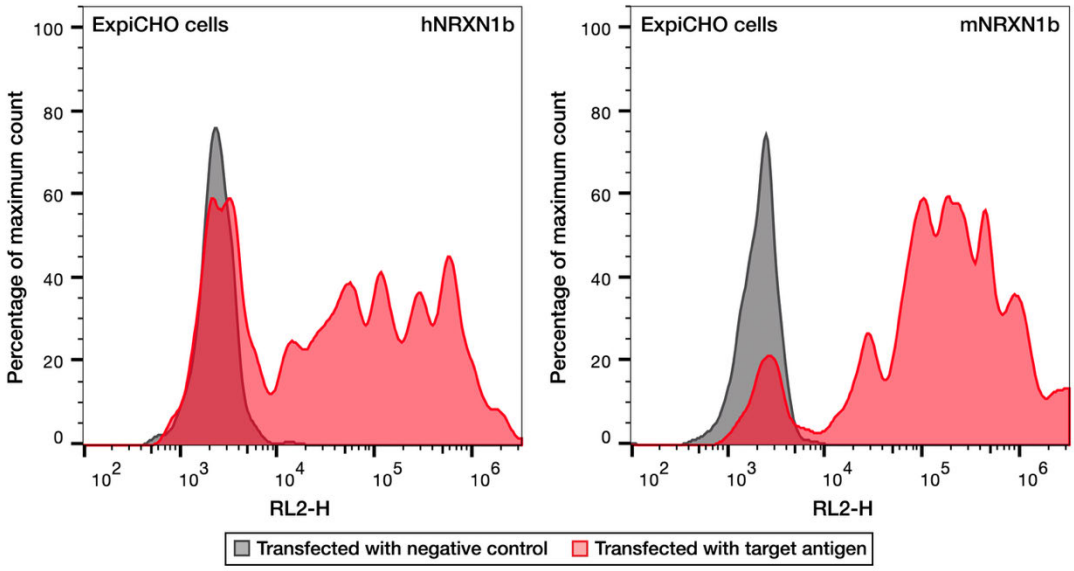
Community Data*

Application	Lab	Reference
IHC	James Trimmer, Ph.D., NeuroMab	https://doi.org/10.57733/addgene.wkw7h6

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

Applications

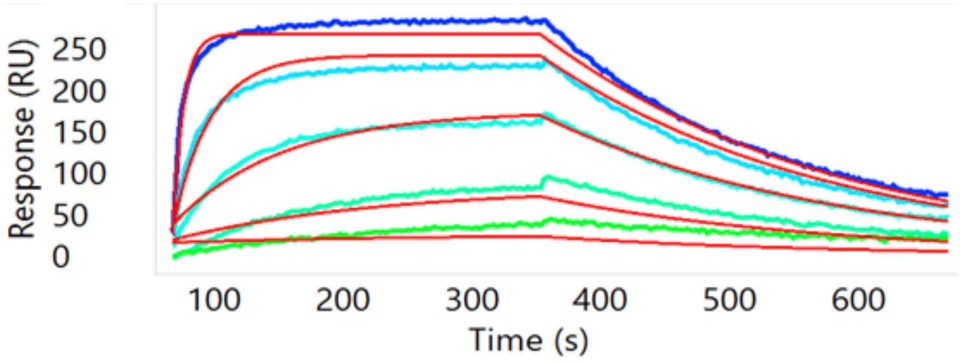
Flow cytometry



Anti-Neurexin-1-beta [IPI-NRXN1b.35] (Addgene #273805) recognizes mouse and human NRXN1b in flow cytometry. Histogram from FACS analysis on ExpiCHO cells transfected with human or mouse NRXN1b (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) NRXN1b were labeled with Anti-Neurexin-1-beta [IPI-NRXN1b.35] 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.rmgf43

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 NRXN1b showed reactivity towards human and mouse NRXN1b with observed EC_{50} values of 0.05 nM and 1 nM for human and mouse NRXN1b, respectively.

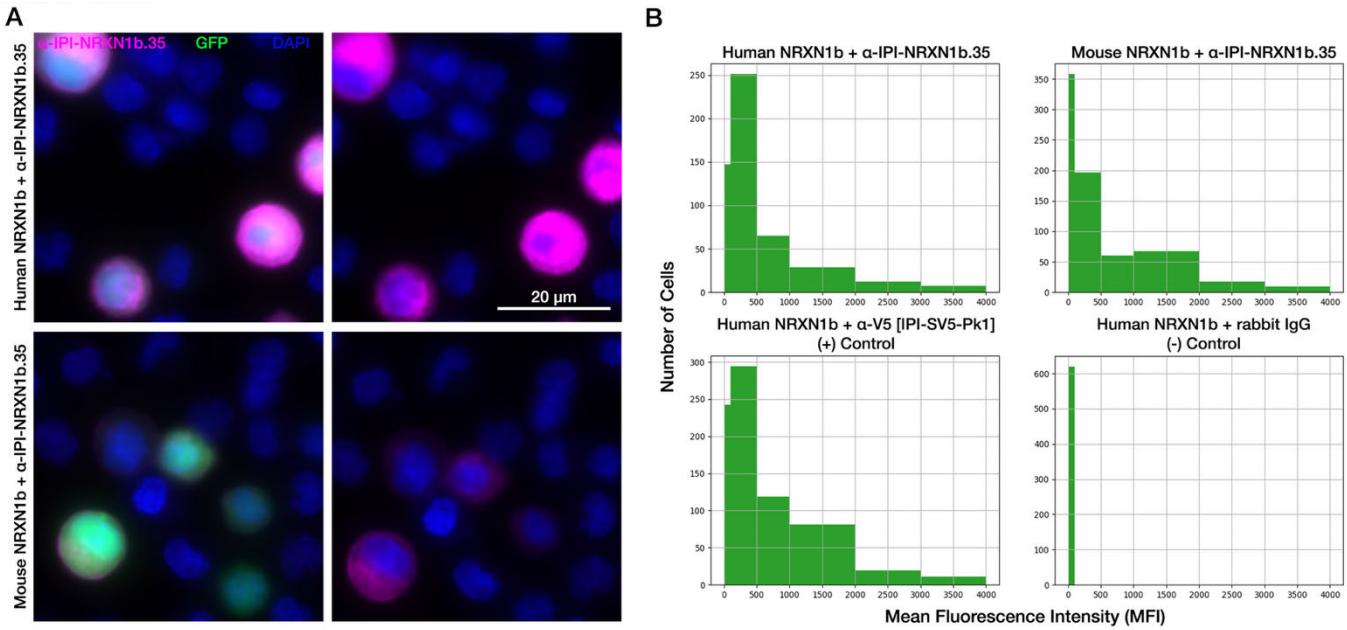
Surface Plasmon Resonance (SPR)



	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	R_{max} (RU)	Res. sd
IPI-NRXN1b.35	3.0 x 10^5	4.4 x 10^{-3}	1.5 x 10^{-8}	278	10.9

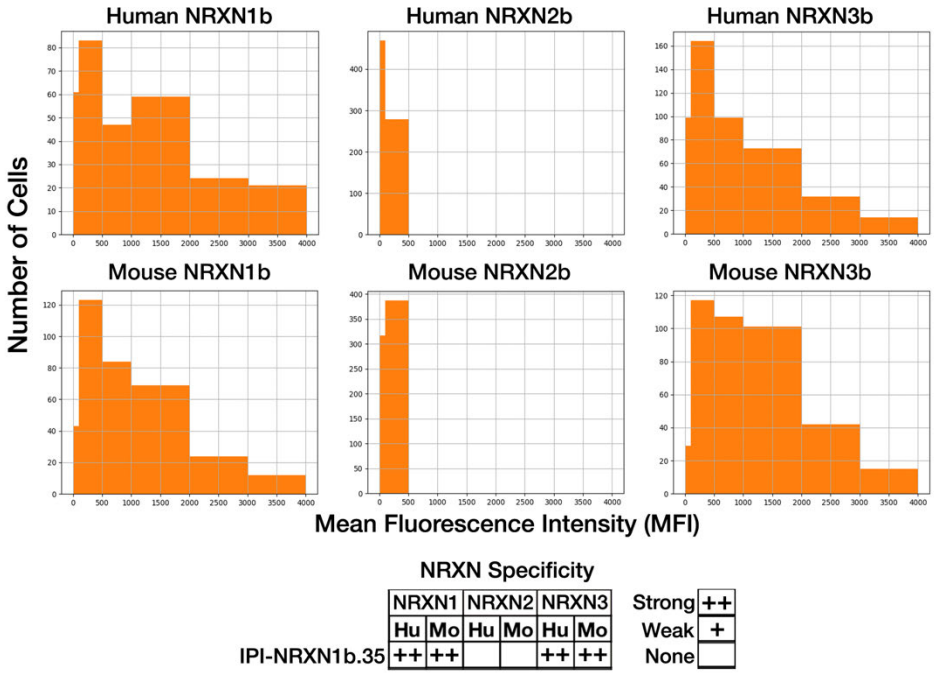
Surface Plasmon Resonance (SPR) kinetics analysis of the interaction between Anti-Neurexin-1-beta [IPI-NRXN1b.35] and mouse NRXN1b. SPR binding kinetics were measured on a Carterra LSA using HC30M chips (Carterra, cat. #4279) at 25°C. Goat anti-rabbit IgG Fc (Jackson ImmunoResearch, cat. #111-005-046) was immobilized via amine coupling, and test antibodies were captured using a 96-channel print-head. Antigens (400nM to five lower concentrations, 2-fold dilutions) were injected in antigen buffer (20mM HEPES pH7.4, 150mM NaCl, 1mM $CaCl_2$, 1mM $MgCl_2$, 0.005% Tween80) with 300s 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.

Immunofluorescence (IF) – Species Reactivity



Anti-Neurexin-1-beta [IPI-NRXN1b.35] (Addgene #273805) recognizes mouse and human NRXN1b in immunofluorescence. A) Immunofluorescence (IF) of ExpiCHO cells transfected with human (top) and mouse (bottom) NRXN1b. 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-NRXN1b.35, GFP (transfection control) and DAPI) while the right images only show IPI-NRXN1b.35 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-NRXN1b.35 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 4095 (saturation). NRXN1b.35 staining of human (left) and mouse (right) NRXN1b is shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-NRXN1b.35 was used at 1 ug/mL (1:1000 dilution). doi: https://doi.org/10.57733/addgene.y6p77c

Immunofluorescence (IF) – Target Specificity



NRXN Specificity

	NRXN1		NRXN2		NRXN3	
	Hu	Mo	Hu	Mo	Hu	Mo
IPI-NRXN1b.35	++	++			++	++

Strong	++
Weak	+
None	

Anti-Neurexin-1-beta [IPI-NRXN1b.35] (Addgene #273805) recognizes mouse and human NRXN1b and NRXN3b in immunofluorescence. 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-NRXN1b.35 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 4095 (saturation). IPI-NRXN1b.35 staining of human and mouse variants of each NRXNb family member is compared on the top and bottom rows. To test family-wide cross-reactivity, IPI-NRXN1b.35 was used at 1 ug/mL (1:1,000 dilution). doi: https://doi.org/10.57733/addgene.4dfaa2

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

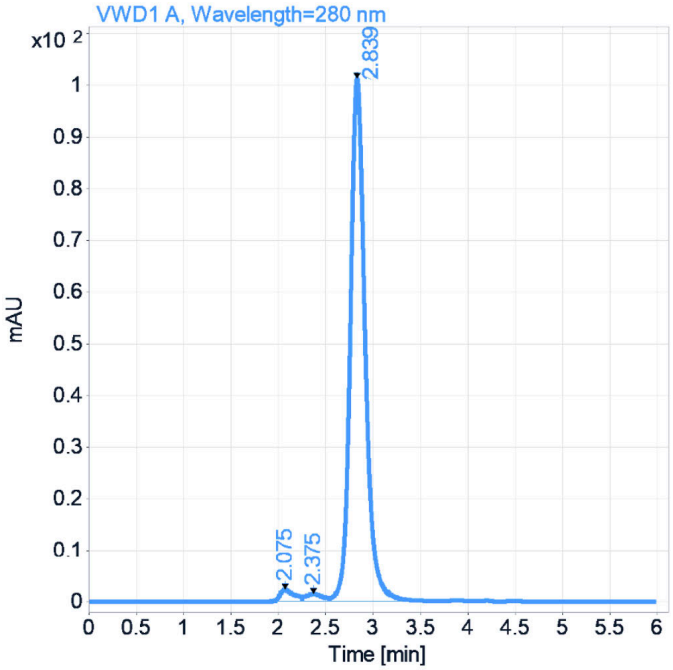
LC-MS: Intact mass analysis via LC-MS methods allows for confirmation antibody mass, and to identify any product-related variants such as glycosylation. Before conducting intact mass analysis via LC-MS, the antibody was reduced to its heavy chain (HC) and light chain (LC). This process allows for confirmation 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-NRXN1b.35	49726.57	49730.90	4.33	23817.44	23817.03	-0.41

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-NRXN1b.35	2.839	0.1815	1102.3513	101.2178	96.4539	Pass

Antigen Details

Immunogen design:

cDNA of Human Neurexin-1-beta with N-terminal FLAG and C-terminal His- and Avi-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:

>Human NRXN1b (AA: 50-397)
DYKDDDDKGSGASSLGAHHIHHFHGSSKHHSVPIAIYRSPASLRGGHAGTTYIFSKGGGQITYKWPPND
RPSTRADRLAIGFSTVQKEAVLVRVDSSSGLGDYLELHIHQGKIGVKFNVGTDDIAIEESNAIINDGKYHVV
RFTRSGGNATLQVDSWPVIERYPAGNNDNERLAIARQRIPYRLGRVVDEWLLDKGRQLTIFNSQATIIIGG
KEQGQPFQGQLSGLYYNGLKVLNMAAENDANIAIVGNVRLVGEVPSSMTTESTATAMQSEMSTSIMETT
TTLATSTARRGKPPTKEPISQTTDDILVASAECPSDDEDIDPCEPSSGGLANPTRAGGREPYPGSAEVIRE
SSSTTGSGGLNDIFEAQKIEWHEGSGHHHHHHHH

Sequence information:

HUGO:	8008
Uniprot:	P58400
Refseq:	NM_001330092.2

Structural information:

Topology:	Single-pass type I membrane protein
PDB IDs:	3B3Q;5Z8Y;6NID
Alphafold:	AF-P58400-F1

Expression profiles:

Human Protein Atlas ENSG00000179915

References

1. Z. Anderson, H. Li, T. Riedel, H. Zhu and D. Moshinsky. (2025). Flow Cytometry for Anti-Neurexin-1-beta [IPI-NRXN1b.35]. Addgene. https://doi.org/10.57733/addgene.rmgf43
2. A. Morano, T. Riedel, and D. Moshinsky. (2025). ICC/IF for Anti-Neurexin-1-beta [IPI-NRXN1b.35] in binding assay. Addgene. https://doi.org/10.57733/addgene.y6p77c
3. A. Morano, T. Riedel, and D. Moshinsky. (2025). ICC/IF for Anti-Neurexin-1-beta [IPI-NRXN1b.35] in specificity assay. Addgene. https://doi.org/10.57733/addgene.4dfaa2
4. J. Trimmer. (2025). IHC for Anti-Neurexin-1-beta [IPI-NRXN1b.35]. Addgene. https://doi.org/10.57733/addgene.wkw7h6

How to cite this antibody:
Anti-Neurexin-1-beta [IPI-NRXN1b.35] - from Institute for Protein Innovation (IPI) (Addgene #237805; http://n2t.net/addgene:237805; RRID: AB_3678704).

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