Product Datasheet

Anti-Netrin-1 [IPI-NTN1.43]

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Overview

Antigen	Netrin-1
Immunogen	Purified recombinant fragment of Human Netrin-1, corresponding to AA: 39-453.
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-NTN1.43
RRID	AB_3698383
IPI ID	TAB0010440-013-006
Specificity	Netrin-1; Does not recognize other netrins
Species reactivity	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.14duwj
IF – Binding	1 µg/mL	Positive	https://doi.org/10.57733/addgene.4a5jmb
IF – Specificity	0.1 µg/mL	Positive	https://doi.org/10.57733/addgene.num6if

‡ Not suitable for WB application.

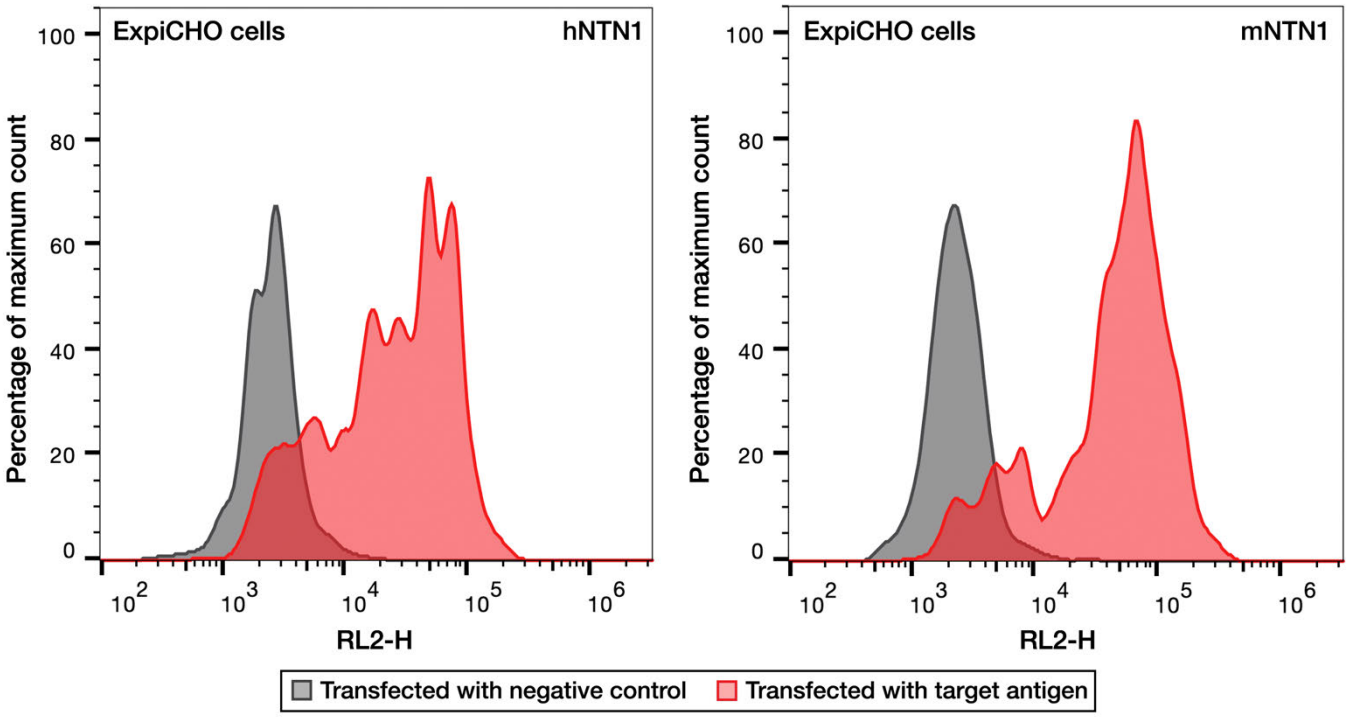
Community Data*

Application	Lab	Reference
IHC	Samantha Butler, Ph.D., UCLA	https://doi.org/10.57733/addgene.amfit7
IP	YCharOS	https://doi.org/10.57733/addgene.qhitqd

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

Applications

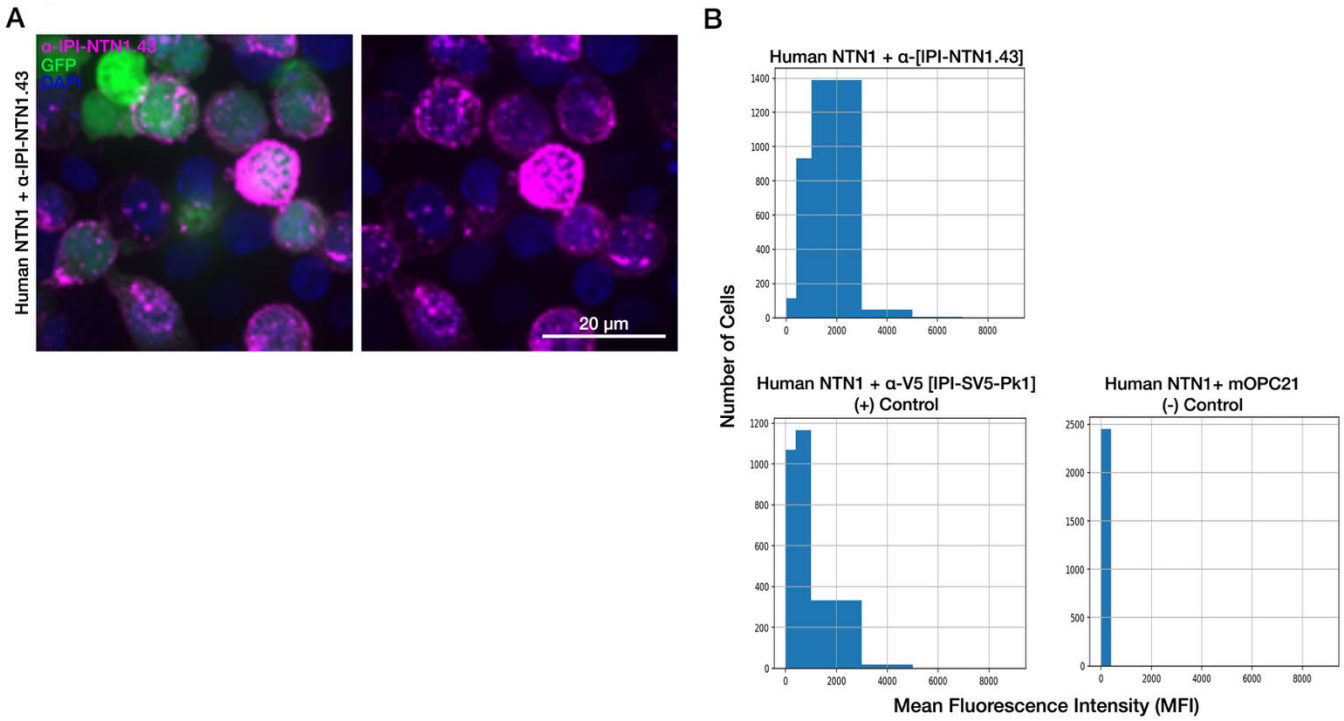
Flow cytometry



Anti-Netrin-1 [IPI-NTN1.43] (Addgene #241894) recognizes human and mouse Netrin-1. Histogram from FACS analysis on ExpiCHO cells transfected with human or mouse Netrin-1 (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) Netrin-1 were labeled with Anti-Netrin-1 [IPI-NTN1.43] and Alexa Fluor 647 F(ab')2 goat anti-rabbit IgG, Fc fragment specific (Jackson ImmunoResearch, 111-606-046). Labeled cells were analyzed with an Intellicyt iQue Screener Plus flow cytometer. Histograms were generated for the 15 ug/mL antibody concentration and normalized to mode using FlowJo™ v10. doi: https://doi.org/10.57733/addgene.14duwj

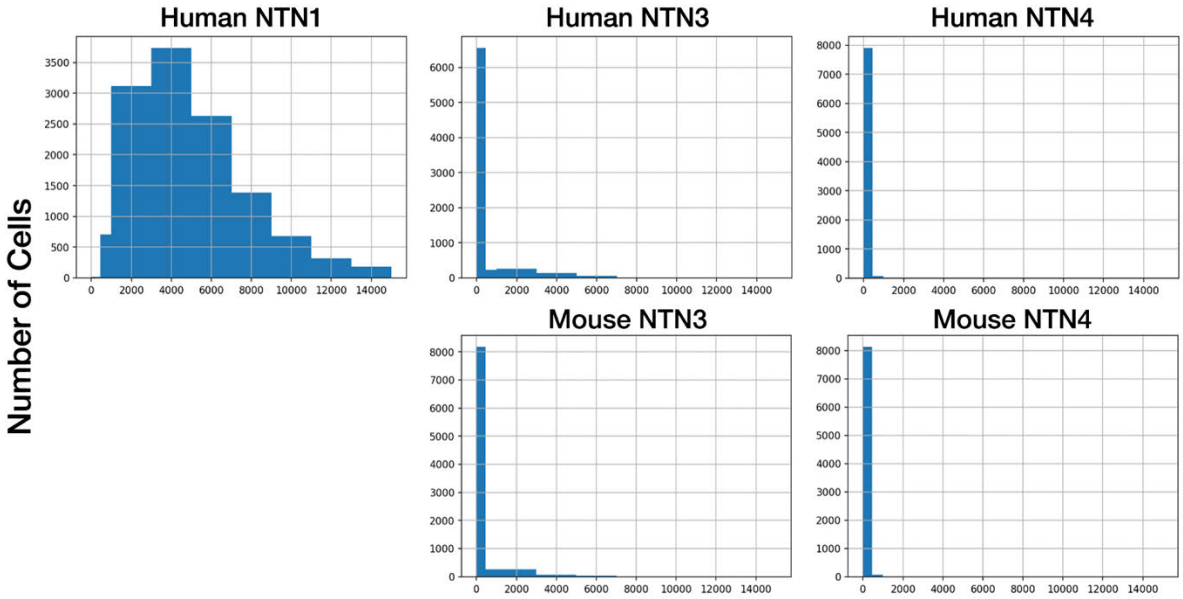
EC_{50} (data not shown): A fourteen-point titration of antibody concentrations, ranging from 660 nM (0.1 mg/mL) to 4.42 pM with a 1:2.5 dilution factor, against human and mouse Netrin-1 showed reactivity towards human and mouse Netrin-1 with observed EC_{50} values of 6.84 nM and 3.087 nM for human and mouse Netrin-1, respectively.

Immunofluorescence (IF) – Species Reactivity



Anti-Netrin-1 [IPI-NTN1.43] (Addgene #241894) shows binding to human Netrin 1. A) Immunofluorescence (IF) of ExpiCHO cells transfected with human Netrin 1 stained with IPI-NTN1.43 (magenta). Confocal images taken at 40X magnification on the ImageXpress confocal HT.ai microscope. Scale bar = 20 uM. 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-NTN1.43 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 10000 (saturation). IPI-NTN1.43 staining of human Netrin 1 is shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-NTN1.43 was used at 1 ug/mL (1:1,000 dilution). doi: https://doi.org/10.57733/addgene.4a5jmb

Immunofluorescence (IF) – Target Specificity



NTN Specificity	NTN1		NTN3		NTN4	
	Hu	Mo	Hu	Mo	Hu	Mo
IPI-NTN1.43	++					

Strong	++
Weak	+
None	

Anti-Netrin-1 [IPI-NTN1.43] (Addgene #241894) shows binding only to human Netrin 1. Each graph depicts the combined quantification of multiple images of the same transfected cells taken at 10X magnification. GFP- or 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-NTN1.43 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 100 (background fluorescence) to 16000 (saturation). IPI-NTN1.43 staining of human and mouse variants of each netrin family member is compared on the top and bottom rows. To test family-wide cross-reactivity, IPI-NTN1.43 was used at 0.1 ug/mL (1:10,000 dilution). doi: https://doi.org/10.57733/addgene.num6if

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

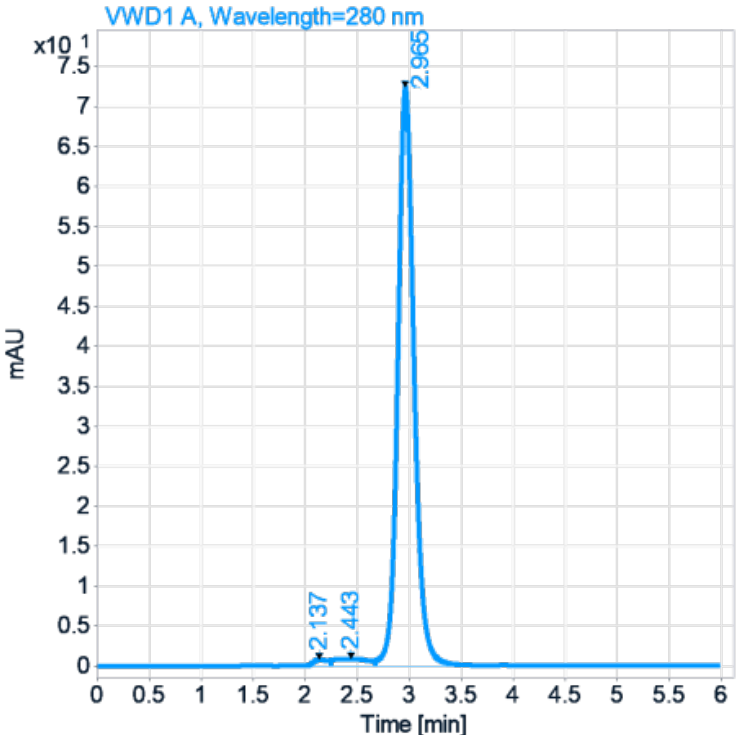
LC-MS: Intact mass analysis via LC-MS methods allows for confirmation antibody mass, and to identify any product-related variants such as glycosylation. Before conducting intact mass analysis via LC-MS, the antibody was reduced to its heavy chain (HC) and light chain (LC). This process allows for confirmation 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-NTN1.43	49955.44	49963.20	7.76	22618.98	22618.11	-0.87

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-NTN1.43	2.965	0.1816	788.3342	72.3503	96.4926	Pass

Antigen Details

Immunogen design:

cDNA of Human Netrin-1 with 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 Netrin-1 (AA: 39-453):
DPCSDENGHPRRCIPDFVNAAFGKDVRVSSTCGRPPARYCVVSERGEERLRSCHLCNASDPKKAHPPA
FLTDLNNPHNLTCWQSENYLQFPHNVTLTLSLGKKFEVTYVSLQFCSPRPESMAIYKSMDYGRTWVPFQ
FYSTQCRKMYNRPHRAPITKQNEQEAVCTDSHTDMRPLSGGLIAFSTLDGRPSAHDFDNSPVLQDWVT
ATDIRVAFSRLHTFGDENEDDSELARDSYFYAVSDLQVGGRCKCNGHAARCVRDRDDSLVCDCRHNTA
GPECDRCKPFHYDRPWQRATAREANECVACNCNLHARRCRFNMELYKLSGRKSGGVCLNCRHNTAG
RHCHYCKEGYYRDMGKPITHRKACKACDCHPVGAAGKTCNQTTGQCPCKDGVTGITCNRCAKGYQQ
SRSPIAPCIKGSGEDQVDPRLIDGKGSGHHHHHHHHHHGSGGLNDIFEAQKIEWHE

Sequence information:

HUGO:	8029
Uniprot:	O95631
Refseq:	NM_004822.3

Structural information:

Topology:	Secreted; soluble and membrane bound forms
PDB IDs:	4URT;6FKQ;7NDG;7NE0;7NE1
Alphafold:	AF-O95631-F1

Expression profiles:

Human Protein Atlas	ENSG00000065320

References

1. Z. Anderson, H. Li, T. Riedel, H. Zhu and D. Moshinsky. (2025). Flow Cytometry for Anti-Netrin-1 [IPI-NTN1.43]. Addgene. https://doi.org/10.57733/addgene.14duwj
2. A. Morano, T. Riedel, and D. Moshinsky. (2025). ICC/IF for Anti-Netrin-1 [IPI-NTN1.43] in binding assay. Addgene. https://doi.org/10.57733/addgene.4a5jmb
3. A. Morano, T. Riedel, and D. Moshinsky. (2025). ICC/IF for Anti-Netrin-1 [IPI-NTN1.43] in specificity assay. Addgene. https://doi.org/10.57733/addgene.num6if
4. Y. Mercado-Ayon and S. Butler. (2025). IHC for Anti-Netrin-1 [IPI-NTN1.43]. Addgene. https://doi.org/10.57733/addgene.amfit7
5. R. Ayoubi , S. González Bolívar and C. Laflamme. (2025). IP for Anti-Netrin-1 [IPI-NTN1.43] in MCF7 culture medium. Addgene. https://doi.org/10.57733/addgene.qhitqd
6. R. Ayoubi, M. Fotouhi, C. Alende, S. González Bolívar, K. Southern, C. Laflamme (2025). A guide to selecting high-performing antibodies for S1PR1 (UniProt ID: P21453) for use in western blot, immunoprecipitation, and immunofluorescence. F1000Res. 2025 Jan 24;13:792. doi: https://doi.org/10.12688/f1000research.153244.3

How to cite this antibody:
Anti-Netrin-1 [IPI-NTN1.43] - from Institute for Protein Innovation (IPI) (Addgene #241894; http://n2t.net/addgene:241894; RRID: AB_3698383).

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