Product Datasheet

EAAT1/GLAST-1/SLC1A3 Antibody - BSA Free
NB100-1869

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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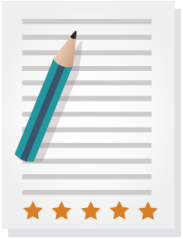
Updated 4/13/2025 v.20.1



NB100-1869

EAAT1/GLAST-1/SLC1A3 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	60 kDa

Product Description

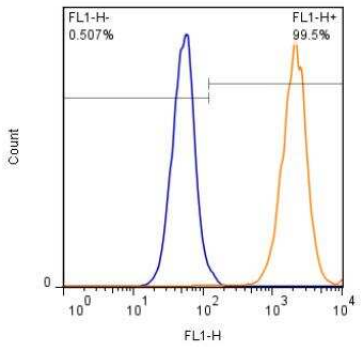
Host	Rabbit
Gene ID	6507
Gene Symbol	SLC1A3
Species	Human, Mouse, Rat
Immunogen	A synthetic peptide made to a C-terminal portion of the rat SLC1A3 protein (between residues 500-542) [Uniprot: P24942]

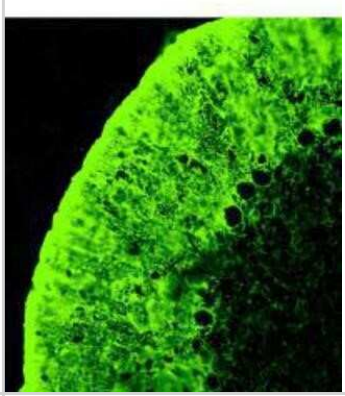
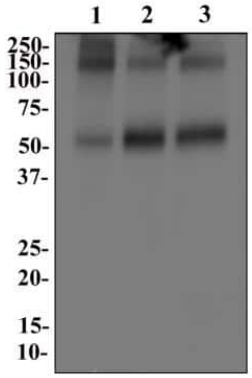
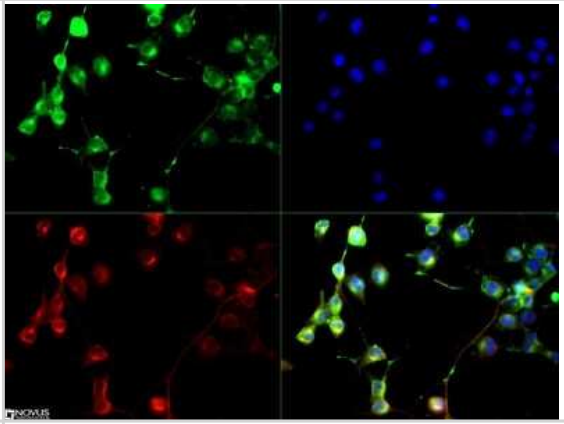
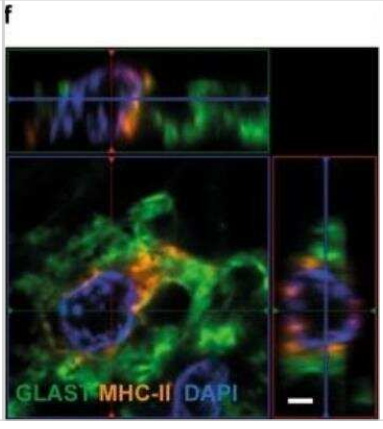
Product Application Details

Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 2 ug/ml, Simple Western, Flow Cytometry 1:200-1:500, ELISA 1:100 - 1:2000, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunohistochemistry-Paraffin 1:50-1:500, Immunohistochemistry-Frozen 1:10-1:500, Flow (Intracellular) 1:500

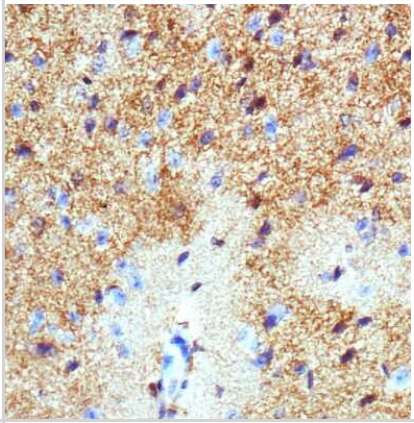
Images

Flow (Intracellular): EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - Intracellular staining of HEK293 cells (1 x 10^6 cells/mL) with SLC1A3 antibody (orange) stained at a dilution of 1:500. Detected with a GtxRb Dylight 488 secondary. Shown with the secondary control (blue).

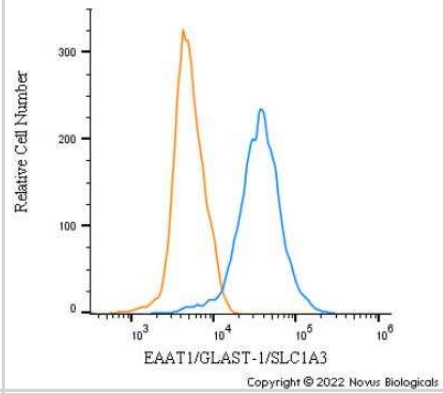


Immunohistochemistry: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - Staining of SLC1A3 in rat cerebellum sections	
Western Blot: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - Analysis of SLC1A3 in 1. Human brain 2. Mouse brain and 3. Rat brain whole cell lysates.	
Immunocytochemistry/Immunofluorescence: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - SLC1A3 antibody was tested at 1:250 in Neuro2A cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red). Image objective 40X.	
Immunocytochemistry/Immunofluorescence: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - Human astrocytes express MHC-II in brain tissue from PD patients and healthy controls. The aSYN pathology was confirmed in the PD cases by immunostaining of aSYN and phosphorylated aSYN (p-aSYN). Astrocytes labeled with EAAT1/GLAST-1/SLC1A3 are expressing high levels of MHC-II, as well as Iba1-labeled microglia. Image collected and cropped by CiteAb from the following publication (https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-020-01776-7), licensed under a CC-BY license.	

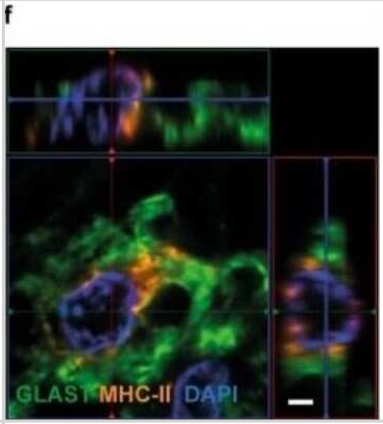
Immunohistochemistry-Paraffin: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - Analysis of SLC1A3 on mouse brain.



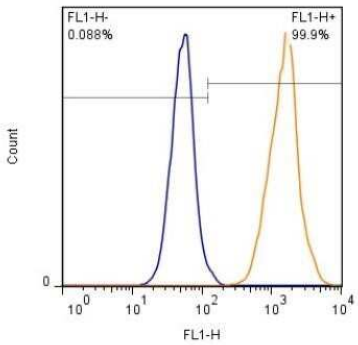
Flow Cytometry: EAAT1/GLAST-1/SLC1A3 Antibody - BSA Free [NB100-1869] - An intracellular stain was performed on rat FR cells with EAAT1/GLAST-1/SLC1A3 Antibody NB100-1869 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).

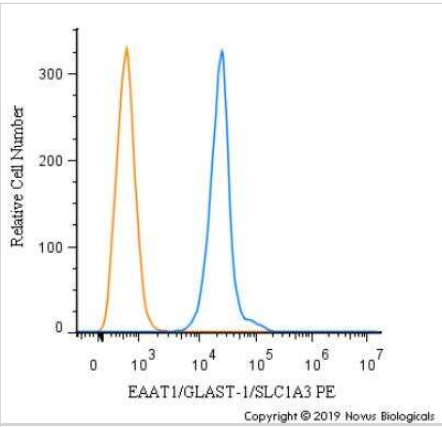
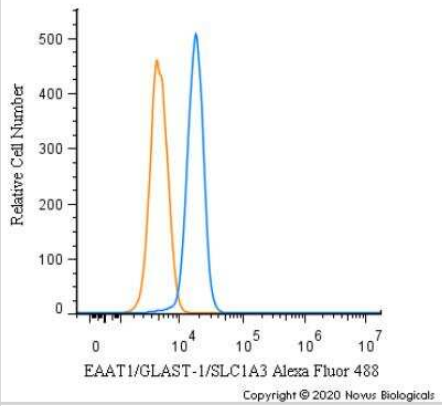
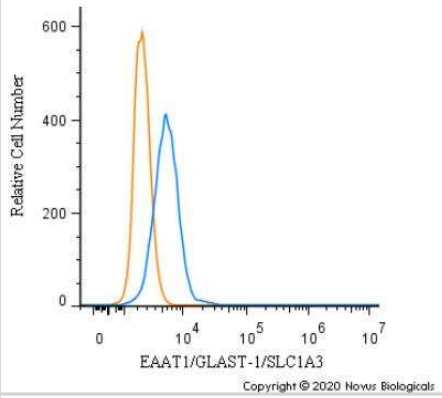
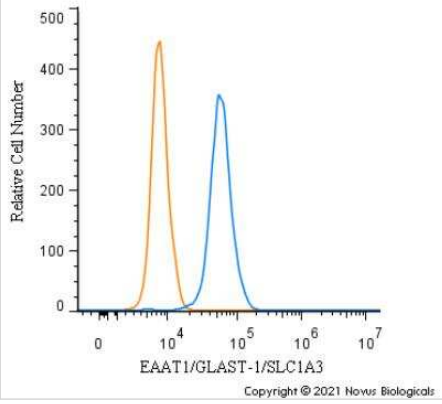


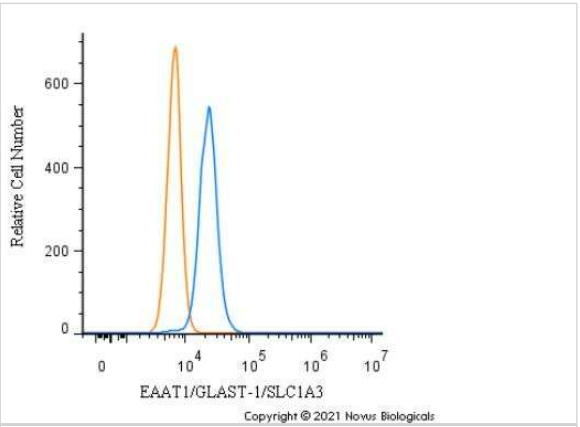
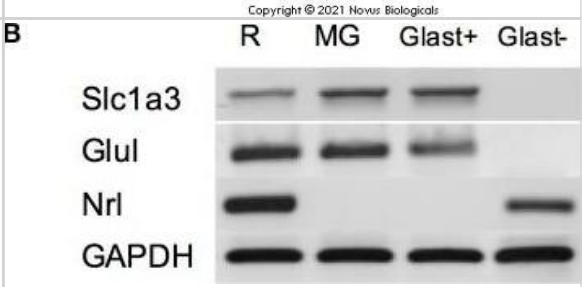
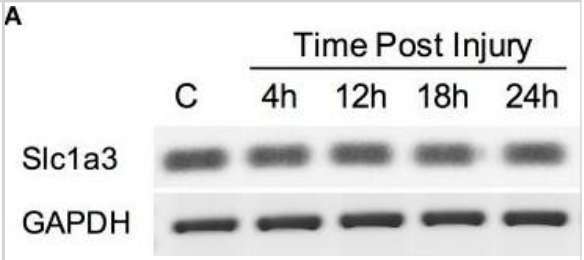
Immunocytochemistry/Immunofluorescence: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - Human astrocytes express MHC-II in brain tissue from PD patients and healthy controls. The aSYN pathology was confirmed in the PD cases by immunostaining of aSYN and phosphorylated aSYN (p-aSYN). Astrocytes labeled with EAAT1/GLAST-1/SLC1A3 are expressing high levels of MHC-II, as well as Iba1-labeled microglia. Image collected and cropped by CiteAb from the following publication (https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-020-01776-7), licensed under a CC-BY license.



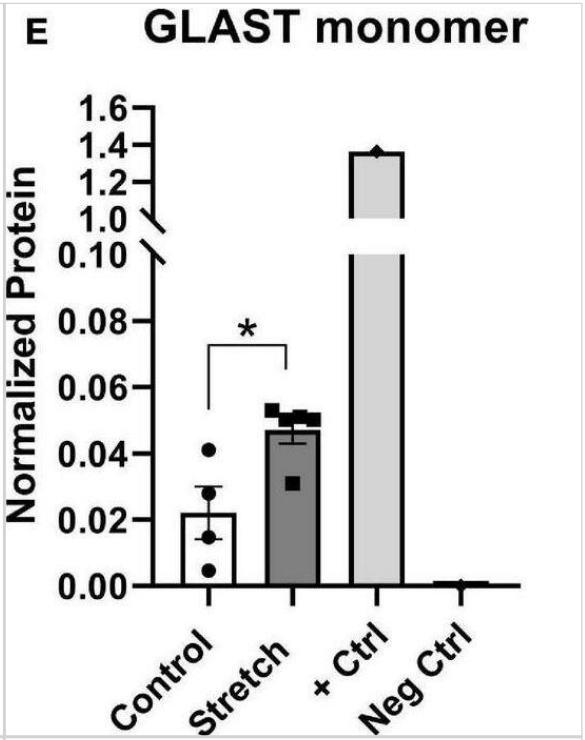
Flow (Intracellular): EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - Staining of HEK293 cells (1 x 10^6 cells/mL) with AF488 conjugated EAAT-1 antibody (orange) stained at a dilution of 1:500. Shown with rIgG (AF488) isotype control (blue).



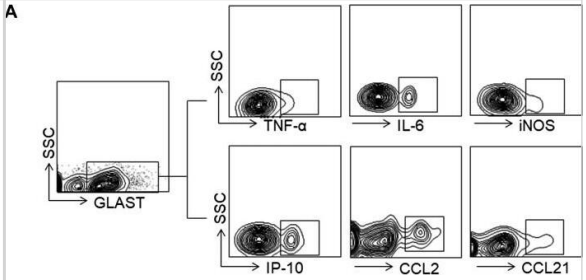
Flow Cytometry: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - An intracellular stain was performed on Hek293 cells with EAAT1/GLAST-1/SLC1A3 antibody NB100-1869PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.	
Flow Cytometry: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - An intracellular stain was performed on Hek293 cells with EAAT1/GLAST-1/SLC1A3 Antibody NB100-1869AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.	
Flow Cytometry: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - An intracellular stain was performed on U937 cells with SLC1A3 Antibody NB100-1869 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Flow Cytometry: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - An intracellular stain was performed on Caco-2 cells with SLC1A3 Antibody NB100-1869 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	

Flow Cytometry: EAAT1/GLAST-1/SLC1A3 Antibody [NB100-1869] - An intracellular stain was performed on Neuro2a cells with SLC1A3 Antibody NB100-1869 (blue) and a matched isotype control NBP2-24891 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
Western Blot: EAAT1/GLAST-1/SLC1A3 Antibody - BSA Free [NB100-1869] - Damage response is restricted to MG. (A) RT-PCR analysis for Slc1a3, the gene encoding GLAST, at the indicated times after injury. (B) RT-PCR for MG & a photoreceptor-specific marker (Nrl) in GLAST-positive & negative fractions, after MACS in intact retinas. (C–F) qPCR quantification of Oct4, Nanog, Lin28, & Dnmt3b expression levels at the indicated time after injury in MACS GLAST-positive & negative fraction; C, intact retina as control (Student's t-test; ***p < 0.001; **p < 0.01; *p < 0.05). Image collected & cropped by CiteAb from the following publication (http://journal.frontiersin.org/article/10.3389/fnins.2016.00523/full), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: EAAT1/GLAST-1/SLC1A3 Antibody - BSA Free [NB100-1869] - Damage response is restricted to MG. (A) RT-PCR analysis for Slc1a3, the gene encoding GLAST, at the indicated times after injury. (B) RT-PCR for MG & a photoreceptor-specific marker (Nrl) in GLAST-positive & negative fractions, after MACS in intact retinas. (C–F) qPCR quantification of Oct4, Nanog, Lin28, & Dnmt3b expression levels at the indicated time after injury in MACS GLAST-positive & negative fraction; C, intact retina as control (Student's t-test; ***p < 0.001; **p < 0.01; *p < 0.05). Image collected & cropped by CiteAb from the following publication (http://journal.frontiersin.org/article/10.3389/fnins.2016.00523/full), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

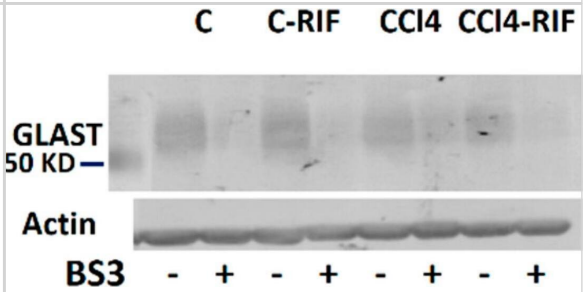
Simple Western: EAAT1/GLAST-1/SLC1A3 Antibody - BSA Free [NB100-1869] - Protein changes in ONHAs corroborate bioenergetics data. (A) Glucose transporter-1 protein levels in Stretched ONH astrocytes are significantly higher than Control (*p = 0.0225, n = 7 Control, n = 8 Stretch). Retinal lysate from a 2 month-old mouse was used as a positive control for each protein analyzed, while negative control was the signal obtained when no primary antibody was included in the capillary. (B) Lactate dehydrogenase-A, the astrocyte-specific isoform of the enzyme that catalyzes the interconversion of pyruvate & lactate, has equivalent protein levels in Control & Stretch ONH astrocytes. (C) Glucose-6-phosphate dehydrogenase, the enzyme that shunts glucose into the pentose phosphate pathway, is no different in Control & Stretch ONH astrocytes. (D) Glutamine synthetase, the enzyme that synthesizes glutamine from glutamate, is no different in Control & Stretch ONH astrocytes. (E) The monomeric form of glutamate-aspartate transporter (GLAST) has significantly higher protein levels in the Stretch as compared to the Control ONH astrocytes (p = 0.020; n = 4 Control, n = 5 Stretch). (F) GLAST dimer protein levels are no different in Control & Stretch ONH astrocytes. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35992925), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Flow Cytometry: EAAT1/GLAST-1/SLC1A3 Antibody - BSA Free [NB100-1869] - Depletion of microglia promotes astrocyte responses following MPTP treatment. C57BL/6 mice received PLX3397 or vehicle for 21 d prior to saline or MPTP administration (intraperitoneal injection). Mice continued to receive PLX3397 or vehicle until the experiments ended. At d 7 after saline or MPTP injection, single cell suspensions were prepared from substantia nigra & striatal tissues. A) Flow cytometry plots show the gating strategy for astrocytes (GLAST+) & their expression of TNF-α, IL-6, iNOS, IP-10, CCL2, & CCL21. B, C) Bar graphs show the effects of microglial depletion on the expression of TNF-α, IL-6, iNOS, IP-10, CCL2, & CCL21 in astrocytes at d 7 after MPTP treatment. All data are presented as means ± sem; n = 9 mice/group. *P < 0.05, **P < 0.01. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29401614), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: EAAT1/GLAST-1/SLC1A3 Antibody - BSA Free [NB100-1869] - Analysis of extracellular levels of GABA & Glutamate & membrane expression of their transporters. Extracellular levels of (A,C) GABA & glutamate were analyzed by microdialysis at 4 weeks. Membrane expression of (B) glutamate transporter GLAST & (D,E) GABA transporters GAT1 & GAT3 were analyzed using BS3 cross-linker. Values are expressed as percentage of control rats & are mean ± SEM of 12 rats per group. One-way ANOVA with Tukey's test for GAT-1 ($F(3,27) = 4.41$, $p < 0.05$), GAT-3 ($F(3,41) = 3.27$, $p < 0.05$) & glutamate ($F(3,36) = 3.00$, $p < 0.05$) & Welch's ANOVA with Dunnett's test for GABA ($W(3,19) = 3.67$, $p < 0.05$) & GLAST ($W(3,13) = 12.07$, $p < 0.001$) were performed to compare all groups. Values significantly different from control rats are indicated by asterisks, from CCl4 rats by a, & from C-RIF rats by b. * $p < 0.05$, a $p < 0.05$, b $p < 0.05$. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34440206), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Pappenhagen N, Yin E, Morgan AB et al. Stretch stress propels glutamine dependency and glycolysis in optic nerve head astrocytes Frontiers in neuroscience 2022-08-05 [PMID: 35992925]

Spitznagel BD, Buchanan RA, Consoli DC et Al. Acute manganese exposure impairs glutamatergic function in a young mouse model of Alzheimer's disease Neurotoxicology 2023-03-10 [PMID: 36621467]

Napit PR, Ali MH, Shakya M et al. NLRX1 Enhances Glutamate Uptake and Inhibits Glutamate Release by Astrocytes Cells 2019-04-30 [PMID: 31052241]

Freel BA, Kelvington BA, Sengupta S et al. Sterol dysregulation in Smith-Lemli-Opitz syndrome causes astrocyte immune reactivity through microglia crosstalk Disease models & mechanisms 2022-12-01 [PMID: 36524414]

C Beretta, E Svensson, A Dakhel, M Zyśk, J Hanrieder, D Sehlin, W Michno, A Erlandsson Amyloid-β deposits in human astrocytes contain truncated and highly resistant proteoforms. Molecular and cellular neurosciences 2024-01-18 [PMID: 38244652]

Agarwal D, Dash N, Mazo KW et al. Human retinal ganglion cell neurons generated by synchronous BMP inhibition and transcription factor mediated reprogramming NPJ Regenerative medicine 2023-09-29 [PMID: 37773257] (ICC/IF, Human)

Details:
Dilution 1:100

Huang M, Tallon C, Zhu X et al. Microglial-Targeted nSMase2 Inhibitor Fails to Reduce Tau Propagation in PS19 Mice Pharmaceutics 2023-09-21 [PMID: 37765332] (Block/Neutralize, Immunocytochemistry/ Immunofluorescence)

Konstantinidis E, Portal B, Mothes T et al. Intracellular deposits of amyloid-beta influence the ability of human iPSC-derived astrocytes to support neuronal function Journal of Neuroinflammation 2023-01-03 [PMID: 36593462]

Konstantinidis E, Dakhel A, Beretta C, Erlandsson A Long-term effects of amyloid-beta deposits in human iPSC-derived astrocytes Molecular and cellular neurosciences 2023-03-11 [PMID: 36907531] (Immunocytochemistry/ Immunofluorescence, Human)

Sandau US, McFarland TJ, Smith SJ et al. Differential Effects of APOE Genotype on MicroRNA Cargo of Cerebrospinal Fluid Extracellular Vesicles in Females With Alzheimer's Disease Compared to Males Frontiers in cell and developmental biology 2022-04-27 [PMID: 35573689] (WB, Human)

Picciolini, S;Mangolini, V;Rod□, F;Montesano, A;Arnaboldi, F;Liuzzi, P;Mannini, A;Bedoni, M;Gualerzi, A; Multiplexing Biosensor for the Detection of Extracellular Vesicles as Biomarkers of Tissue Damage and Recovery after Ischemic Stroke International journal of molecular sciences 2023-04-27 [PMID: 37175644] (Human)

Zy?k M, Beretta C, Naia L et al. Amyloid-? accumulation in human astrocytes induces mitochondrial disruption and changed energy metabolism Journal of neuroinflammation 2023-02-20 [PMID: 36803838] (Immunocytochemistry/ Immunofluorescence)

More publications at http://www.novusbio.com/NB100-1869

Procedures

Western blot protocol for SLC1A3 antibody (NB100-1869)
Western Blot Protocol
1. Perform SDS-PAGE on protein samples to be analyzed, loading 10-40 ug of total protein per lane.
2. Electro-blot the proteins to a suitable membrane (PVDF or Nitrocellulose) according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or a similar product) to assess transfer success. Mark molecular weight standards where appropriate.
4. Thoroughly rinse the membrane of stain with TBST.
5. Incubate the membrane in blocking buffer (5% non-fat milk in TBST or 5% BSA in TBST) as appropriate, for 60 minutes.
6. Dilute the SLC1A3 primary antibody as appropriate in blocking buffer and incubate for 60 minute at room temperature to overnight at 4 degrees C with gently shaking.
7. Wash the membrane in TBST three times for 10 minutes each.
8. Incubate the membrane in the appropriate secondary antibody prepared in blocking buffer (as per manufacturer's instructions) and incubate for 60 minutes at room temperature.
9. Wash the membrane in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
10. Incubate the membrane in the appropriate detection reagent in accordance with the manufacturer's instructions and image the blot.
Note: Tween-20 can be added to the blocking, wash and antibody dilution buffers to a final concentration of 0.05-0.1%.

Immunohistochemistry-Paraffin protocol for SLC1A3 antibody (NB100-1869)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 60 minutes at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 degrees C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry/Immunofluorescence protocol for SLC1A3 antibody (NB100-1869)

Culture cells to appropriate density on suitable glass coverslips in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and gently add 10% formalin to cover the cells. Fix at room temperature for 5-10 minutes.
2. Remove the formalin and add 0.5% Triton-X 100 in TBS to permeabilize the cells. Incubate for 5-10 minutes.
3. Remove the permeabilization buffer and add wash buffer (PBS or PBS with 0.1% Tween-20). Be sure to not let the cells dry out. Gently wash three times for 10 minutes.
4. Alternatively, cells can be fixed with -20 degrees C methanol for 10 minutes. Remove the methanol and rehydrate in PBS for 10 minutes before proceeding.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum for 60 minutes at room temperature.
6. Add primary antibody at the appropriate dilution and incubate at room temperature for 60 minutes or at 4 degrees C overnight.
7. Remove primary antibody and replace with wash buffer. Gently wash three times for 10 minutes.
8. Add secondary antibody at the appropriate dilution. Incubate for 60 minutes at room temperature.
9. Remove antibody and replace with wash buffer. Gently wash three times for 10 minutes.
10. Nuclei (DNA) can be stained with 4',6' diamino phenylindole (DAPI) at 0.1 ug/ml, or coverslips can be directly mounted in media containing DAPI.
11. Cells can now be viewed with a fluorescence microscope.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow proper laboratory procedures for the disposal of formalin.

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