Product Datasheet

xCT Antibody - BSA Free
NB300-317

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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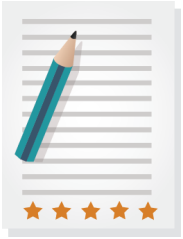
Updated 4/13/2025 v.20.1



NB300-317

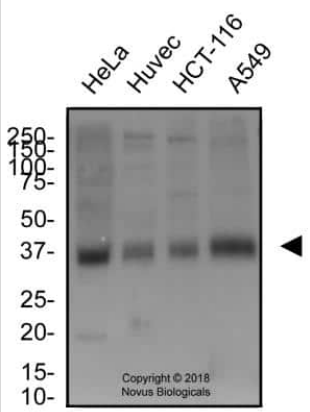
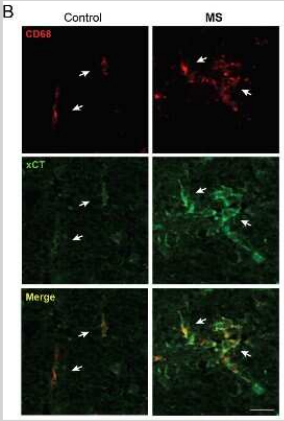
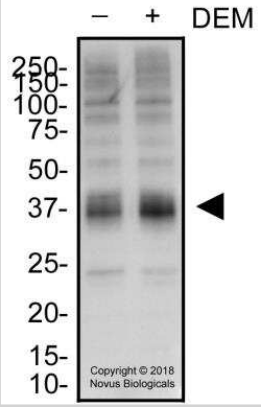
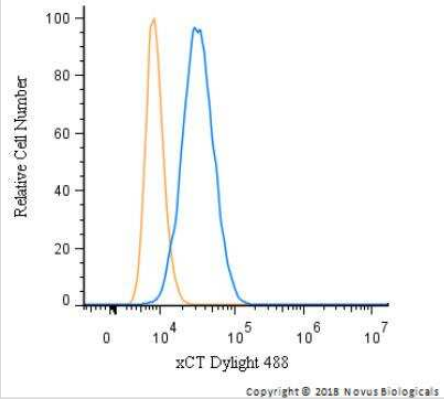
xCT Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1 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	55 kDa

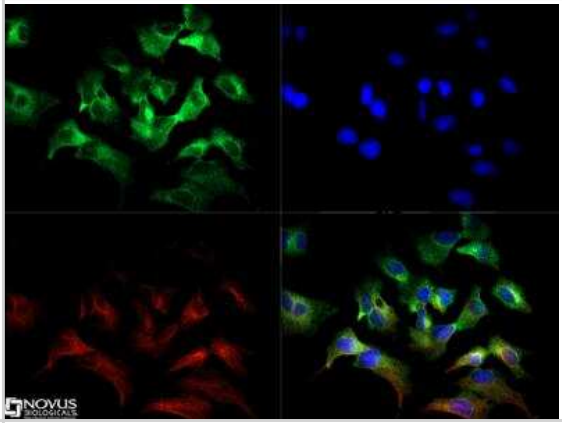
Product Description	
Host	Rabbit
Gene ID	23657
Gene Symbol	SLC7A11
Species	Human, Mouse, Rat
Reactivity Notes	Immunogen displays the following percentage of sequence identity for non-tested species: orangutan (82%). Mouse reactivity reported in scientific literature (PMID: 30279737).
Immunogen	This xCT Antibody was prepared from a synthetic peptide made to a region within the N-terminus of the mouse xCT protein (between residues 1-50). [Uniprot: Q9WTR6]

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 0.5-2 ug/ml, Simple Western, Flow Cytometry 1 - 5 ug/ml, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:100 - 1:1000, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen 1:10-1:500. Use reported in scientific literature (PMID 21639880), Immunoblotting reported in scientific literature (PMID 28185919)
Application Notes	In Western blot, a band is observed at ~35 kDa. In ICC/IF, membrane and ER staining was visualized in HepG2 cells.

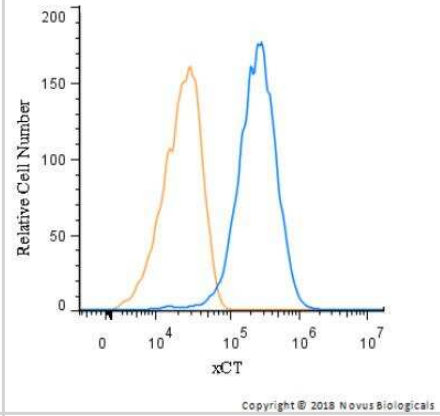
Images

Western Blot: xCT Antibody [NB300-317] - Total protein from human HeLa, Huvec, HCT-116 and A549 cells was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-xCT in block buffer and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.	
Immunohistochemistry-Paraffin: xCT Antibody [NB300-317] - xCT expression is enhanced in CD68+ cells from MS spinal cord. CD68+ cells (arrows) show enhanced xCT expression in MS patients as compared to controls. CD68+ macrophages are round shaped and form clusters in MS patients, whereas in controls, CD68+ cells appear isolated and long shaped. Scale bar = 50 um. Image collected and cropped by CiteAb from the following publication (https://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-8-63), licensed under a CC-BY license.	
Western Blot: xCT Antibody [NB300-317] - Total protein from Human HeLa cells treated with and without 0.1 mM Diethyl Maleate for 24 hours was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-xCT in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Note the increase in xCT expression with treatment.	
Flow Cytometry: xCT Antibody [NB300-317] - An intracellular stain was performed on HeLa cells with NB300-317G (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 DyLight 488.	

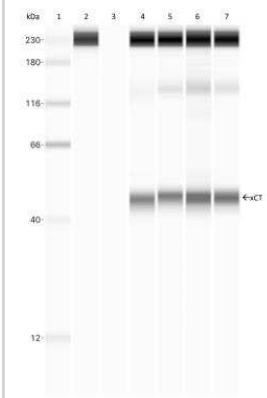
Immunocytochemistry/Immunofluorescence: xCT Antibody [NB300-317] - xCT antibody was tested in HepG2 cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



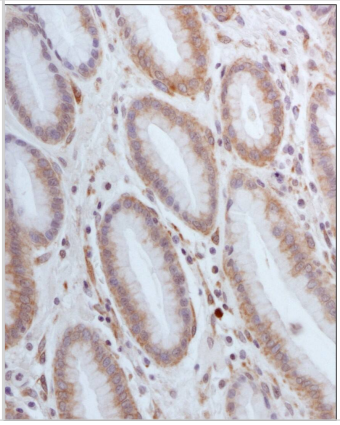
Flow Cytometry: xCT Antibody [NB300-317] - An intracellular stain was performed on HeLa with NB300-317 and a matched isotype control. Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG APC-conjugated Secondary Antibody (F0111, R&D Systems).



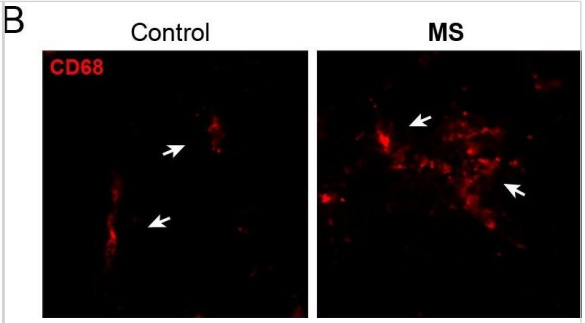
Simple Western: xCT Antibody [NB300-317] - (1) ladder, (2) no lysate + xCT, 100ug/ml, (3) human brain frontal cortex membrane lysate,no primary antibody, (4-7) human brain lysates, 0.03mg/ml, xCT, 100ug/ml.



Analysis of a FFPE tissue section of human stomach using 1:200 dilution of xCT antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



Immunocytochemistry/ Immunofluorescence: xCT Antibody - BSA Free [NB300-317] - xCT expression is enhanced in CD68+ cells from MS spinal cord. A. Triple immunofluorescence staining for xCT (green), CD68 (red) & Hoechst 33258 (blue) in spinal cord of control (left) & MS patients (right). A high expression of xCT was detected in CD68+ infiltrating macrophages (arrows) associated with blood vessels, which are virtually absent in controls. Note that overall xCT expression is enhanced in MS tissue. B. CD68+ cells (arrows) show enhanced xCT expression in MS patients as compared to controls. CD68+ macrophages are round shaped & form clusters in MS patients, whereas in controls, CD68+ cells appear isolated & long shaped. Scale bar = 50 µm. Image collected & cropped by CiteAb from the following publication (https://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-8-63), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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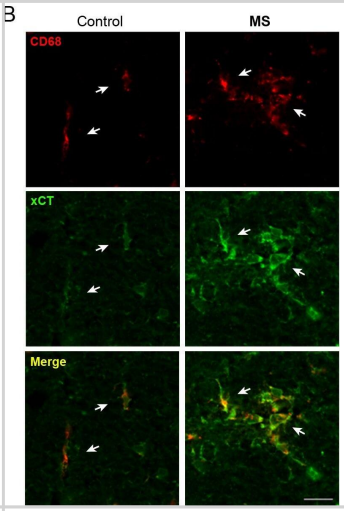
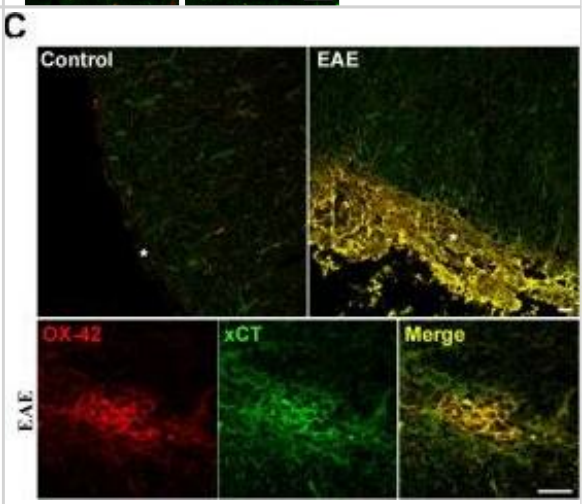


Immunocytochemistry/ Immunofluorescence: xCT Antibody - BSA Free [NB300-317] - xCT expression is increased in the CNS of rats with EAE. A. Histogram showing the neurological score during the course of acute EAE induced in Lewis rats by immunization with myelin basic protein. The peak of neurological disability was at day 14 post-immunization, which was selected for obtaining tissue samples. B. xCT mRNA (left) & protein (right) expression in spinal cord from control & acute EAE rats, as assessed by qPCR & Western blot analysis. Data are referred to mean expression level of controls (n = 5-6). C. Double immunofluorescence for xCT (green) & OX-42 (red), a marker of microglia & infiltrating macrophages. OX42+ cells express high xCT levels in acute EAE. Both meninges (asterisk in top) & infiltrating cells (bottom) in inflammatory foci show high levels of xCT in rat spinal cord with EAE as compared to controls. D. Microglial cells (OX42+ cells) of EAE rats have higher xCT levels in spinal cord than controls. Notice the difference between resting microglia in control rats, with ramified morphology (arrows in control) & microglia in EAE showing round shaped morphology, characteristic of its activated state (arrows in EAE). Scale bar = 20 µm.E. xCT mRNA (left) & protein (right) expression in spinal cord from control & chronic EAE mice, assessed by qPCR & Western blot analysis. Data are referred to mean expression level of controls (n = 5). Image collected & cropped by CiteAb from the following publication (https://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-8-63), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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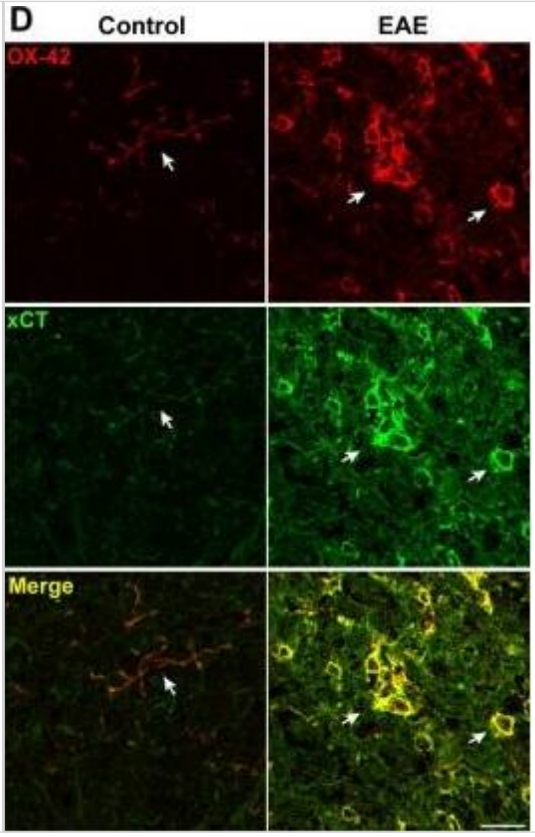

Publications

Martinez R 3rd, Huang W, Samadani R et al. Mechanistic Analysis of an Extracellular Signal-Regulated Kinase 2-Interacting Compound that Inhibits Mutant BRAF-Expressing Melanoma Cells by Inducing Oxidative Stress Journal of Pharmacology and Experimental Therapeutics 2021-01-01 [PMID: 33109619] (Block/Neutralize)

Zhang HF, Hughes CS, Li W et al. Proteomic Screens for Suppressors of Anoikis Identify IL1RAP as a Promising Surface Target in Ewing Sarcoma Cancer Discovery 2021-11-01 [PMID: 34021002]

Davidson-Hunt A Supplemental glutamine to restore glutathione homeostasis in the aging mouse liver Thesis 2022-01-01 (WB, Mouse)

Details:
Dilution used in WB 1:500

Wang J, Wang Y, Liu Y et al. Ferroptosis, a new target for treatment of renal injury and fibrosis in a 5/6 nephrectomy-induced CKD rat model Cell death discovery 2022-03-22 [PMID: 35318301] (IHC-P, Rat)

Long Y, Tao H, Karachi A et al. Dysregulation of glutamate transport enhances Treg function that promotes VEGF blockade resistance in glioblastoma Cancer Res. 2019-11-13 [PMID: 31723000] (IHC-Fr, Mouse)

Huang M, Lin Y, Chang C et al. RGS4 deficit in prefrontal cortex contributes to the behaviors related to schizophrenia via system xc--mediated glutamatergic dysfunction in mice. Theranostics 2018-09-13 [PMID: 30279737] (WB, Mouse)

Chew SH, Okazaki Y, Akatsuka S et al. Rheostatic CD44 isoform expression and its association with oxidative stress in human malignant mesothelioma. Free Radic. Biol. Med. 2017-02-06 [PMID: 28185919]

Linher-Melville K, Haftchenary S, Gunning P et al. Signal transducer and activator of transcription 3 and 5 regulate system Xc- and redox balance in human breast cancer cells. Mol. Cell. Biochem. 2015-04-21 [PMID: 25896132] (WB, Human)

Pampliega O, Domercq M, Soria FN et al. Increased expression of cystine/glutamate antiporter in multiple sclerosis. J Neuroinflammation. 2011-06-01 [PMID: 21639880] (IHC-Fr, Rat, Human)

Liu, R et al. Cystine-glutamate transporter SLC7A11 mediates resistance to geldanamycin but not to 17-(allylamino)-17-demethoxygeldanamycin. Mol Pharmacol;72(6):1637-46. 2007-12-01 [PMID: 17875604] (WB, Human)

Procedures

Western Blot Protocol for xCT Antibody (NB300-317)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunocytochemistry/Immunofluorescence Protocol for xCT Antibody (NB300-317)
Immunocytochemistry Protocol

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

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Immunohistochemistry-Paraffin Protocol for xCT Antibody (NB300-317)
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 (keep slides in the sodium citrate buffer at all times).

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

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Products Related to NB300-317

NB820-59260	Human Stomach Whole Tissue Lysate (Adult Whole Normal)
NB300-317PEP	xCT Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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