Product Datasheet

xCT Antibody
NB300-318SS

Unit Size: 0.025 ml

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

Reviews: 1 Publications: 20

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB300-318

Updated 6/15/2014 v.20.1

NB300-318SS

xCT Antibody

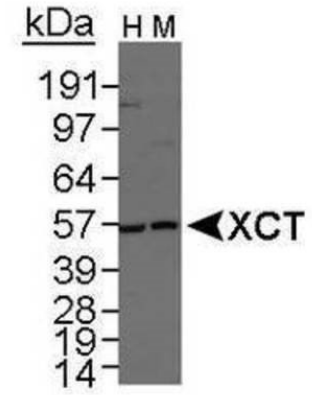
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	57 kDa

Product Description	
Host	Rabbit
Gene ID	23657
Gene Symbol	SLC7A11
Species	Human, Mouse, Rat
Species Reactivity	Human, mouse and rat (PMID 21540084).
Immunogen	A synthetic peptide made to an N-terminal region of the human xCT protein (between residues 1-50) [UniProt Q9UPY5].

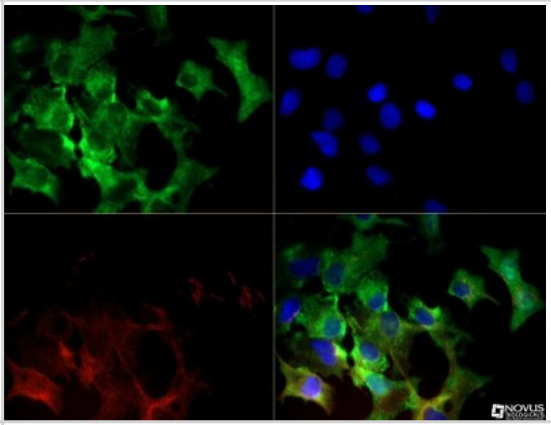
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation (Negative)
Recommended Dilutions	Flow Cytometry 2-3 ug/ml, Immunocytochemistry/Immunofluorescence 1:100-1:1000, Immunohistochemistry 5 u/gml, Immunohistochemistry-Paraffin 5 ug/ml, Immunoprecipitation (Negative), Western Blot 1:1000, Simple Western 10 ug/ml
Application Notes	This xCT antibody is useful for Western blot, Immunocytochemistry/Immunofluorescence, Immunohistochemistry and Flow (PMID 20028852). Immunoprecipitation is not recommended. In Western blot this antibody recognizes a band at ~55 kDa, and in ICC/IF membrane staining was observed in HepG2 cells. Permeablization is recommended prior to performing Flow analysis.In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

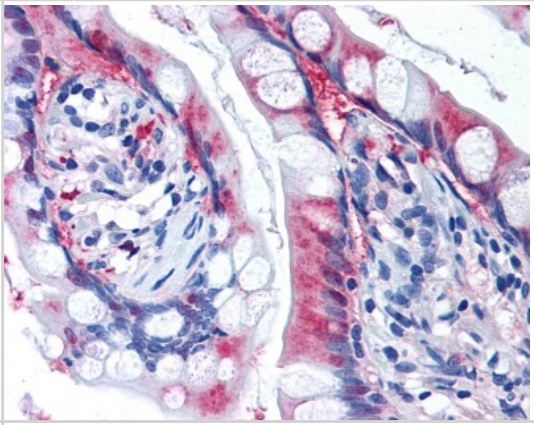
Western Blot: xCT Antibody [NB300-318] - Detection of xCT in total human and mouse stomach lysate, respectively, using NB300-318. 1 minute ECL exposure.



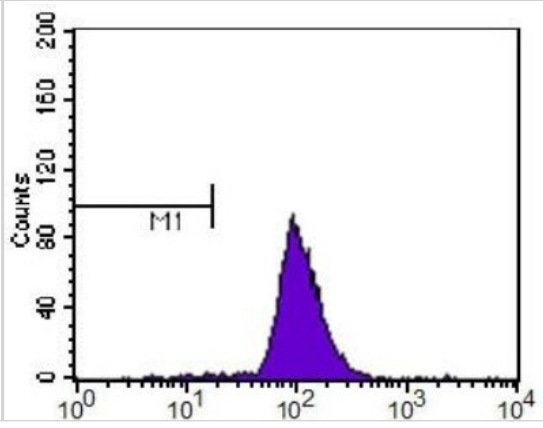
Immunocytochemistry/Immunofluorescence: xCT Antibody [NB300-318] - xCT antibody was tested in HepG2 cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



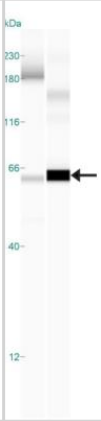
Immunohistochemistry: xCT Antibody [NB300-318] - xCT staining in the absorptive epithelia of small intestinal villi detected using NB300-318.



Flow Cytometry: xCT Antibody [NB300-318] - xCT antibody was tested at 1:400 in HepG2 cells using an Alexa Fluor 488 secondary (shown in purple). M1 is defined by unstained cells.



Simple Western: xCT Antibody [NB300-318] - Simple Western lane view shows a specific band for xCT in 0.5 mg/ml of Human Stomach Cancer (left) and HepG2 (right) lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system. * Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody



Publications

Rao PS, Saternos H, Goodwani S, Sari Y. Effects of ceftriaxone on GLT1 isoforms, xCT and associated signaling pathways in P rats exposed to ethanol Psychopharmacology (Berl.). 2015 Jan 27 [PMID: 25619881] (WB, Rat)

Aal-Aaboda M, Alhaddad H, Osowik F et al. Effects of (R)-(-)-5-methyl-1-nicotinoyl-2-pyrazoline on glutamate transporter 1 and cysteine/glutamate exchanger as well as ethanol drinking behavior in male, alcohol-preferring rats J. Neurosci. Res. 2015 Jan 19 [PMID: 25601490] (WB, Rat)

Martin L, Gardner Lb. et al. Stress-induced inhibition of nonsense-mediated RnA decay regulates intracellular cystine transport and intracellular glutathione through regulation of the cystine/glutamate exchanger SLC7A11. Oncogene. 2014 Nov 17 [PMID: 25399695] (WB, Human)

Yang Y, Yee D. IGF-I Regulates Redox Status in Breast Cancer Cells by Activating the Amino Acid Transport Molecule xC-. Cancer Res. 2014 Apr 15 [PMID: 24686172] (WB, Human)

Fontana JM, Mygatt JG, Conant KL, Parsons CH. Kaposi's Sarcoma-Associated Herpesvirus Subversion of the Anti-Inflammatory Response in Human Skin Cells Reveals Correlates of Latency and Disease Pathogenesis. Journal of Skin cancer 2/17/2014 [PMID: 24701351] (WB, Human)

Drayton RM, Dudziec E, Peter S et al. Reduced expression of microRNA-27a modulates cisplatin resistance in bladder cancer by targeting the cystine/glutamate exchanger SLC7A11. Clin. Cancer Res. 2014 Feb 11 [PMID: 24516043] (WB, Human)

Timmerman LA, Holton T, Yuneva M et al. Glutamine Sensitivity Analysis Identifies the xCT Antiporter as a Common Triple-Negative Breast Tumor Therapeutic Target. Cancer Cell. 2013 Oct 14 [PMID: 24094812] (IHC, Human)

D'Elia M, Patenaude J, Dupras C et al. Burn injury induces the expression of cystine/glutamate transporter (x(c)(-)) in mouse T cells. Immunol Lett. 2009 Aug 15 [PMID: 19576933] (WB, Mouse)

Pei S, Minhajuddin M, Callahan KP et al. Targeting Aberrant Glutathione Metabolism to Eradicate Human Acute Myelogenous Leukemia Cells. J Biol Chem. 2013 Oct 2 [PMID: 24089526] (WB, Human)

Wengrod J, Martin L, Wang D et al. The inhibition of nonsense mediated RNA decay activates autophagy Mol Cell Biol 2013 Mar 18 [PMID: 23508110] (WB, Human)

Baek S, Mueller A, Lim YS et al. (4S)-4-(3-18F-Fluoropropyl)-L-Glutamate for Imaging of xC Transporter Activity in Hepatocellular Carcinoma Using PET: Preclinical and Exploratory Clinical Studies. J Nucl Med 2013 Jan [PMID: 23232273]

Balza E, Castellani P, Delfino L et al. The pharmacologic inhibition of the xc- antioxidant system improves the antitumor efficacy of COX inhibitors in the in vivo model of 3-MCA tumorigenesis Carcinogenesis 2012 Nov 28 [PMID: 23161574]

More publications at http://www.novusbio.com/NB300-318

Procedures

Western blot Protocol specific for xCT antibody (NB300-318)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer 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 manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunocytochemistry/Immunofluorescence Protocol for xCT Antibody (NB300-318)
Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunohistochemistry Protocol for NB300-318 (NB300-318)

Immunohistochemistry

1. Prepare tissue with formalin fixation and by embedding it in paraffin wax.
2. Make 4-mm sections and place on pre-cleaned and charged microscope slides.
3. Heat in a tissue-drying oven for 45 minutes @ 60 degrees Celcius.
4. Deparaffinize the tissues by wash drying the slides in 3 changes of xylene for 5 minutes each @ RT.
5. Rehydrate the tissues by washing the slides in 3 changes of 100% alcohol for 3 minutes each @ RT.
6. Wash the slides in 2 changes of 95% alcohol for 3 minutes each @ RT.
7. Wash the slides in 1 change of 80% alcohol for 3 minutes @ RT.
8. Rinse the slides in gentle running distilled water for 5 minutes @ RT.
9. Perform antigen retrieval by steaming the slides in 0.01M sodium citrate buffer (pH 6.0) @ 99-100 degrees Celcius for 20 minutes.
10. Remove the slides from the heat and let stand in buffer @ RT for 20 minutes.
11. Rinse the slides in 1X TBS-T for 1 minute @ RT.

Do not allow the tissues to dry at any time during the staining procedure

12. Begin the immunostaining by applying a universal protein block for 20 minutes @ RT.
13. Drain protein block from the slides and apply the diluted primary antibody for 45 minutes @ RT.
14. Rinse the slide in 1X TBS-T for 1 minute @ RT.
15. Apply a biotinylated anti-rabbit IgG (H+L) secondary for 30 minutes @ RT.
16. Rinse the slide in 1X TBS-T for 1 minute @ RT.
17. Apply an alkaline phosphatase steptavidin for 30 minutes @ RT.
18. Rinse the slide in 1X TBS-T for 1 minute @ RT.
19. Apply an alkaline phosphatase chromagen substrate for 30 minutes @ RT.
20. Rinse the slide in distilled water for 1 minute @ RT.

This method should only be used if the chromagen substrate is alcohol insoluble (ie: Vector Red, DAB)

21. Dehydrate the tissue by washing the slides in 2 changes of 80% alcohol for 1 minute each @ RT.
22. Wash the slides in 2 changes of 95% alcohol for 1 minute each @ RT.
23. Wash the slides in 3 changes of 100% alcohol for 1 minute each @ RT.
24. Wash the slides in 3 changes of xylene for 1 minute each @ RT.
25. Apply cover slip.

Novus Biologicals USA
8100 Southpark Way, A-8
Littleton, CO 80120
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
novus@novusbio.com

Novus Biologicals Canada
461 North Service Road West, Unit B37
Oakville, ON L6M 2V5
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada@novusbio.com

Novus Biologicals Europe
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: technical@novusbio.com
Orders: orders@novusbio.com
General: novus@novusbio.com

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 guarantee, please visit www.novusbio.com/guarantee.