Product Datasheet

xCT Antibody - BSA Free
NB300-318SS

Unit Size: 0.025 ml

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

Reviews: 3 Publications: 82

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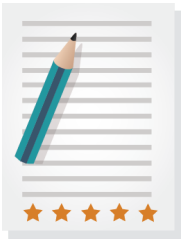
Updated 4/13/2025 v.20.1



NB300-318SS

xCT Antibody - BSA Free

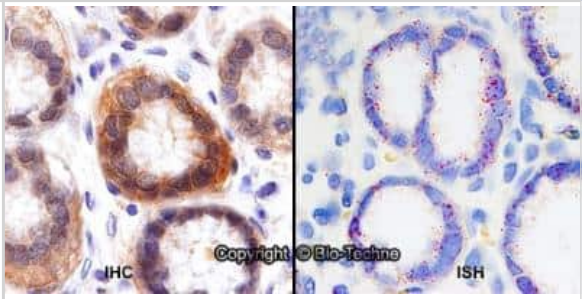
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	23657
Gene Symbol	SLC7A11
Species	Human, Mouse, Rat
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 21540084).
Immunogen	This xCT Antibody was prepared from a synthetic peptide made to an N-terminal region of the human xCT protein (between residues 1-50) [UniProt Q9UPY5].

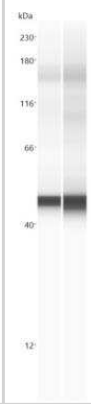
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Dual RNAscope ISH-IHC, Immunoprecipitation (Negative)
Recommended Dilutions	Western Blot 1:1000, Simple Western 10 ug/ml, Flow Cytometry 2-3ug/ml. Use reported in scientific literature (PMID 20028852), Immunohistochemistry 5 u/gml, Immunocytochemistry/ Immunofluorescence 1:100-1:1000, Immunohistochemistry-Paraffin 5 ug/ml, Immunoprecipitation (Negative), Dual RNAscope ISH-IHC
Application Notes	Immunoprecipitation is not recommended. In Western blot this antibody recognizes a band at approx. 35 kDa, and in ICC/IF membrane staining was observed in HeLa 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. See Simple Western Antibody Database for Simple Western validation: Tested in HepG2 lysate, HeLa and HeLa + DEM cell lysates, separated by Size, antibody dilution of 25 ug/mL, apparent MW was 63 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

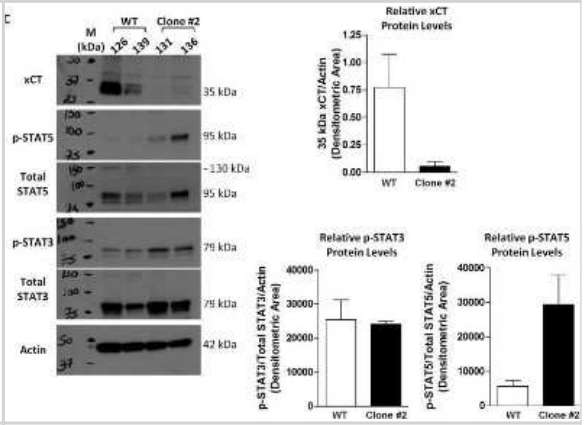
Dual RNAscope ISH-IHC: xCT Antibody [NB300-318] - Formalin-fixed paraffin-embedded tissue sections of human stomach were probed for xCT mRNA (ACD RNAScope Probe, catalog #422688; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using Rabbit Polyclonal (Novus Biologicals catalog # NB300-318) at 0.25ug/mL with 1 hour incubation at room temperature followed by incubation with anti-rabbit IgG VisUCyte HRP Polymer Antibody (Catalog # VC003) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to glandular cells.



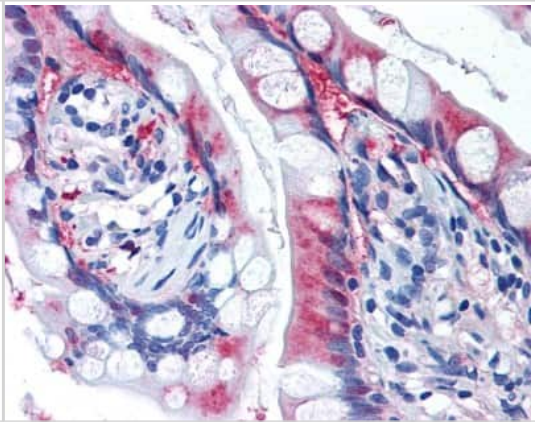
Simple Western: xCT Antibody [NB300-318] - Simple Western lane view shows a specific band for xCT using NB300-318 at 25 ug/ml in HeLa and HeLa + DEM cell lysates. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



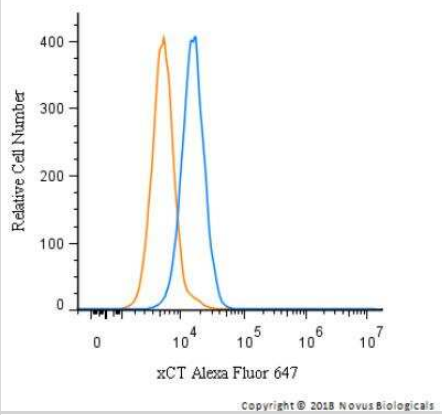
Western Blot: xCT Antibody [NB300-318] - Western blot analysis of protein isolated from subcutaneous tumors derived from in vivo growth of the clones relative to WT-derived tumors revealed that xCT levels remained low, phospho-STAT5 (p-STAT5) levels remained high, and phospho-STAT3 (p-STAT3) levels remained unchanged in the absence of SH-4-54. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0161202), licensed under a CC-BY license.



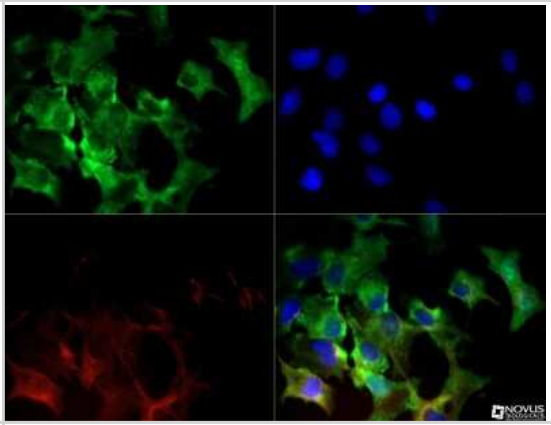
Immunohistochemistry: xCT Antibody [NB300-318] - xCT staining in the absorptive epithelia of small intestinal villi detected using NB300-318.



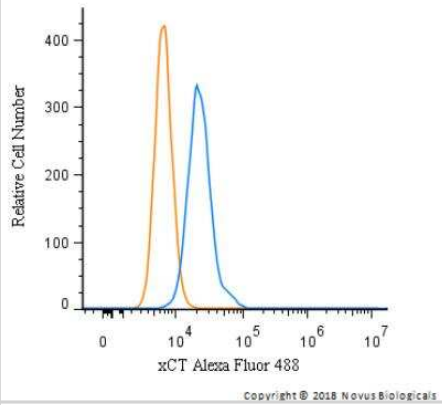
Flow Cytometry: xCT Antibody [NB300-318] - An intracellular stain was performed on HeLa cells with NB300-318AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



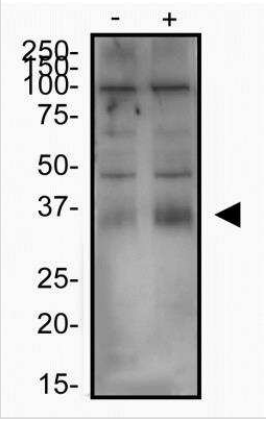
Immunocytochemistry/Immunofluorescence: xCT Antibody [NB300-318] - xCT antibody was tested in HepG2 cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



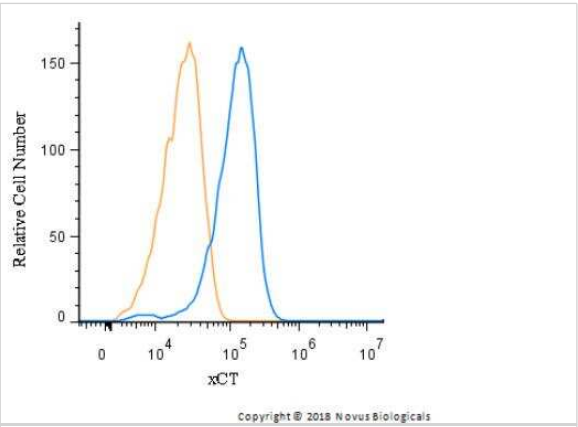
Flow Cytometry: xCT Antibody [NB300-318] - An intracellular stain was performed on HeLa cells with xCT Antibody NB300-318AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



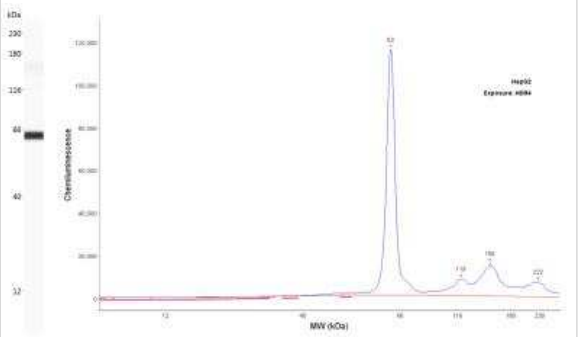
Western Blot: xCT Antibody [NB300-318] - Total protein from Human HeLa cells treated with and without 0.1 mM Diethyl Maleate for 24 hours was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-xCT in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Note the increase in xCT expression with treatment.



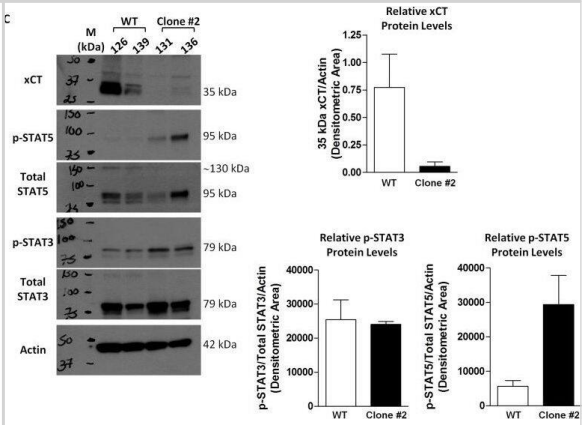
Flow Cytometry: xCT Antibody [NB300-318] - An intracellular stain was performed on HeLa with xCT Antibody NB300-318 and a matched isotype control. Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG APC-conjugated Secondary Antibody (F0111, R&D Systems).



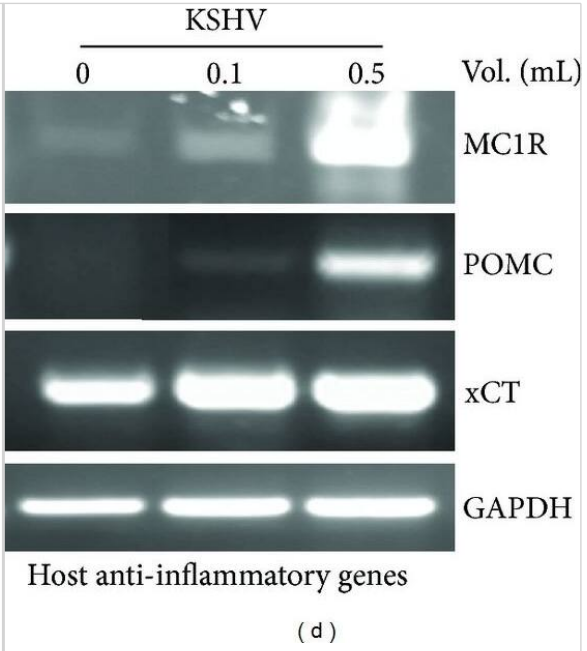
Simple Western: xCT Antibody [NB300-318] - Simple Western lane view shows a specific band for xCT using NB300-318 at 25 ug/mL in HepG2 cell lysates and antibody at 25 ug/mL. Electropherogram image of corresponding Simple Western lane view at WES molecular weight of 63 kDa. Image reported by internal validation.



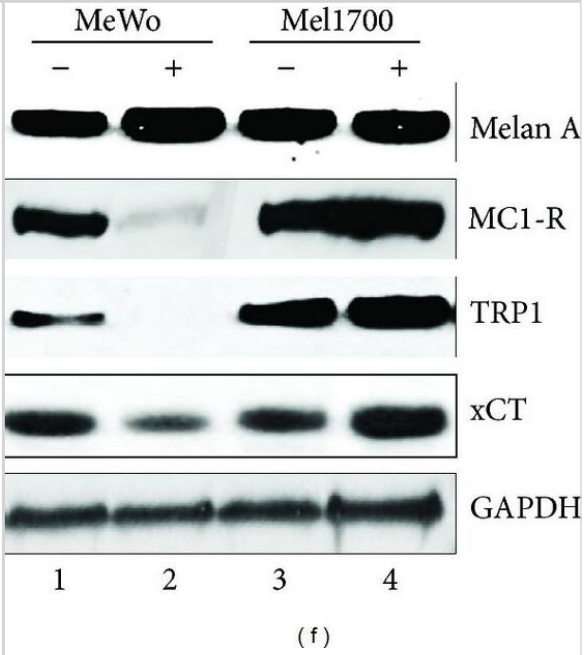
Western Blot: xCT Antibody - BSA Free [NB300-318] - Subcutaneous injection into nude mice revealed that (A) MDA-MB-231 SH-4-54-resistant clone #2 proliferated at a slower rate than its wild-type (WT) counterpart in vivo. (B) qPCR demonstrated that xCT mRNA levels were lower in tumours isolated from animals injected with clone #2 relative to WT cells (2 animals per treatment group). (C) Western blot analysis of protein isolated from subcutaneous tumours derived from in vivo growth of the clones relative to WT-derived tumours revealed that xCT levels remained low, phospho-STAT5 (p-STAT5) levels remained high, & phospho-STAT3 (p-STAT3) levels remained unchanged in the absence of SH-4-54. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0161202), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: xCT Antibody - BSA Free [NB300-318] - A link between KSHV latency & the MC1-R signaling axis in skin-derived cell lines. (a) Western blot analysis of phosphorylated NF-κB p65, MC1-R, & TRP-1 in total cell lysates extracted from MeWo (a) or Mel1700 (b) cells either uninfected (control) or acutely infected with KSHV for 0.3 h, 1 h, 3 h, or 6 h. GAPDH was used as loading control. (c) ImageJ quantitation of the p65 band intensities in (a) & (b) relative to GAPDH controls. (d) Mel1700 cells were infected in 6-well plates with increasing volumes (mL/well) of concentrated supernatant containing infectious KSHV, & total RNA from infected cells was subjected to RT-PCR using primer sets for host anti-inflammatory MC1R, POMC, & SLC7A11. (e) Equal aliquots from the same RNA used in (d) were subjected to RT-PCR analysis for select viral latency & cell growth control genes (i.e., GPCR, LANA, & v-FLIP). (f) Western blot analysis of the melanoma cell marker, Melan A, & anti-inflammatory genes MC1-R, TRP1, & SLC7A11 in total cell lysates of uninfected (−) or chronically infected (+) long-term cultures of MeWo-KSHV & Mel1700-KSHV cells. GAPDH was used as an internal control for both the RT-PCR (e) & western blot assays. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24701351), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: xCT Antibody - BSA Free [NB300-318] - A link between KSHV latency & the MC1-R signaling axis in skin-derived cell lines. (a) Western blot analysis of phosphorylated NF-κB p65, MC1-R, & TRP-1 in total cell lysates extracted from MeWo (a) or Mel1700 (b) cells either uninfected (control) or acutely infected with KSHV for 0.3 h, 1 h, 3 h, or 6 h. GAPDH was used as loading control. (c) ImageJ quantitation of the p65 band intensities in (a) & (b) relative to GAPDH controls. (d) Mel1700 cells were infected in 6-well plates with increasing volumes (mL/well) of concentrated supernatant containing infectious KSHV, & total RNA from infected cells was subjected to RT-PCR using primer sets for host anti-inflammatory MC1R, POMC, & SLC7A11. (e) Equal aliquots from the same RNA used in (d) were subjected to RT-PCR analysis for select viral latency & cell growth control genes (i.e., GPCR, LANA, & v-FLIP). (f) Western blot analysis of the melanoma cell marker, Melan A, & anti-inflammatory genes MC1-R, TRP1, & SLC7A11 in total cell lysates of uninfected (−) or chronically infected (+) long-term cultures of MeWo-KSHV & Mel1700-KSHV cells. GAPDH was used as an internal control for both the RT-PCR (e) & western blot assays. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24701351), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Mi Y, Wei C, Sun L et al. Melatonin inhibits ferroptosis and delays age-related cataract by regulating SIRT6/p-Nrf2/GPX4 and SIRT6/NCOA4/FTH1 pathways Biomedicine & Pharmacotherapy 2023-01-01 [PMID: 36463827]

JW Park, O Kilic, M Deo, K Jimenez-Co, E Demirdizen, H Kim, ? Turcan CIC reduces xCT/SLC7A11 expression and glutamate release in glioma Acta neuropathologica communications, 2023-01-16;11(1):13. 2023-01-16 [PMID: 36647117]

Ku CC, Wuputra K, Kato K et Al. Jdp2-deficient granule cell progenitors in the cerebellum are resistant to ROS-mediated apoptosis through xCT/Slc7a11 activation Sci Rep 2020-03-18 [PMID: 32188872]

Minjung Seo, Yeji Kim, Byong Duk Ye, Sang Hyoung Park, Seog-Young Kim, Jin Hwa Jung, Sung Wook Hwang, Sun Young Chae, Dong Yun Lee, Sang Ju Lee, Seung Jun Oh, Jihun Kim, Ji Young Kim, Sae Jung Na, Misung Kim, Sang-Yeob Kim, Norman Koglin, Andrew W Stephens, Mi-Na Kweon, Dae Hyuk Moon PET Imaging of System x C - in Immune Cells for Assessment of Disease Activity in Mice and Patients with Inflammatory Bowel Disease. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 2022-10-05 [PMID: 35086893]

Cheng Z, Akatsuka S, Li GH et al. Ferroptosis resistance determines high susceptibility of murine A/J strain to iron-induced renal carcinogenesis Cancer Science 2022-01-01 [PMID: 34699654] (Immunohistochemistry, Western Blot)

Tang Y, Luo H, Xiao Q et al. Isoliquiritigenin attenuates septic acute kidney injury by regulating ferritinophagy-mediated ferroptosis Renal Failure 2021-11-18 [PMID: 34791966] (Immunohistochemistry)

Park M, Cho YL, Choi Y et al. Particulate matter induces ferroptosis by accumulating iron and dysregulating the antioxidant system BMB Reports 2023-02-28 [PMID: 36476270] (Block/Neutralize)

Zhou Y, Qian W, Li X, Wei W. NF-?B Inhibitor Myrislignan Induces Ferroptosis of Glioblastoma Cells via Regulating Epithelial-Mesenchymal Transformation in a Slug-Dependent Manner Oxidative Medicine and Cellular Longevity 2023-01-16 [PMID: 36699318] (Block/Neutralize)

Kim HY, Choi YJ, Kim SK et al. Auranofin prevents liver fibrosis by system Xc-mediated inhibition of NLRP3 inflammasome Communications Biology 2021-06-30 [PMID: 34193972] (In vivo assay)

Bao X, Luo X, Bai X et al. Cigarette tar mediates macrophage ferroptosis in atherosclerosis through the hepcidin/FPN/SLC7A11 signaling pathway Free radical biology & medicine 2023-03-16 [PMID: 36933812]

Shi J, Wang QH, Wei X et al. Histone acetyltransferase P300 deficiency promotes ferroptosis of vascular smooth muscle cells by activating the HIF-1?/HMOX1 axis Molecular medicine (Cambridge, Mass.) 2023-07-06 [PMID: 37415103] (WB, Human)

Yan Y, Teng H, Hang Q et al. SLC7A11 expression level dictates differential responses to oxidative stress in cancer cells Nature communications 2023-06-21 [PMID: 37339981] (IHC-P, Mouse)

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

Procedures

Western Blot protocol 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 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.

*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 xCT Antibody (NB300-318)
xCT Antibody:
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 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 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.

Flow (Intracellular) protocol for xCT Antibody (NB300-318)

xCT Antibody:

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeablization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

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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 100% guarantee, please visit www.novusbio.com/guarantee

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