Product Datasheet

SR-BI Antibody - BSA Free
NB400-101

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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NB400-101

SR-BI Antibody - BSA Free

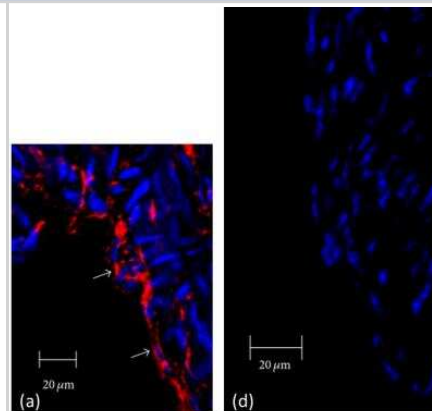
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	82 kDa

Product Description	
Host	Rabbit
Gene Symbol	SCARB1
Species	Human, Mouse, Rat, Bovine, Chinese Hamster, Mustelid, Primate, S. japonicum
Reactivity Notes	Bovine reactivity reported in scientific literature (PMID: 24196350).
Immunogen	A C-terminal peptide containing residues from mouse SR-BI (within residues 450 -509). [Uniprot: Q61009]

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Flow (Intracellular), Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Block/Neutralize, Knockout Validated
Recommended Dilutions	Western Blot 1:1000-1:5000, Simple Western 1:100, Flow Cytometry 1:400. Use reported in scientific literature (PMID 23029167), Immunohistochemistry 2.5-5 ug/ml, Immunocytochemistry/ Immunofluorescence 1:50-1:1000, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 2.5-5 ug/ml, Immunohistochemistry-Frozen reported in scientific literature (PMID 24244566), Immunoblotting reported in scientific literature (PMID 27599291), Flow (Intracellular), Knockout Validated, Block/Neutralize reported in scientific literature (PMID 12119305; 26905520)
Application Notes	In Western blot a band is observed at approx. 82 kDa in tissues that express SR-BI such as liver, ovary and adrenals and to a lesser extent testis, heart and mammary gland. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.

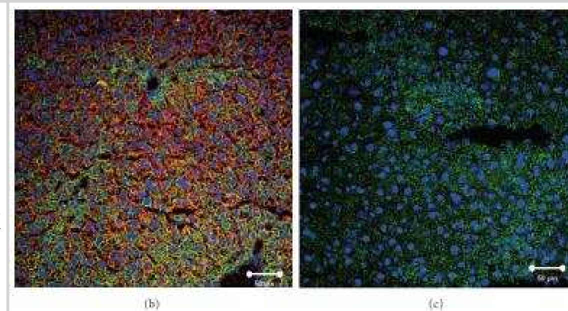
Images

Knockout Validated: SR-BI Antibody [NB400-101] - Immunofluorescent analysis of SR-BI localization in aorta of Tie2-Scarb1 x Scarb1-KO and Scarb1-KO mice. Mouse aorta sections were stained for SR-BI (a and d, red). In several EC stained for SR-B1 red color was present on both apical and basolateral sides. There is no red signal in aorta from Scarb1-KO mice. Image collected and cropped by CiteAb from the following publication (//www.hindawi.com/journals/bmri/2015/607120/) licensed under a CC-BY license.

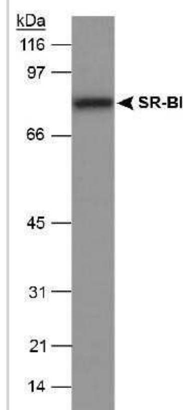


Simple Western: SR-BI Antibody [NB400-101] - Lane view shows a specific band for SR-BI in 0.5 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

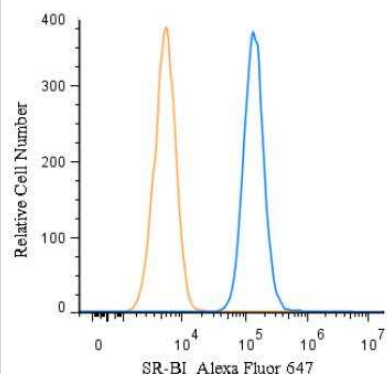
Knockout Validated: SR-BI Antibody [NB400-101] - Immunofluorescent analysis of SR-BI localization in liver of normal, Tie2-Scarb1 x Scarb1-KO, and Scarb1-KO mice. Liver sections from normal (b) and Tie2-Scarb1 x Scarb1-KO mice (c) were stained for SR-BI (red) and cytokeratin 8-18 (green).Merge image for normal mouse (b) demonstrates strong presence of SR-BI in hepatocytes (b, yellow signal) and absence of detectable level of SR-BI protein in liver of Tie2-Scarb1 x Scarb1-KO mice (c). In Scarb1-KO mice there was no detectable level of SR-BI protein (d, staining for SR-BI). Blue = DAPI. Image collected and cropped by CiteAb from the following publication (http://www.hindawi.com/journals/bmri/2015/607120/) licensed under a CC-BY license.



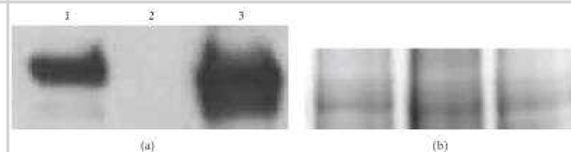
Western Blot: SR-BI Antibody [NB400-101] - Detection of SR-BI in mouse liver lysate (20 ug) using NB 400-101. ECL detection 5 seconds.



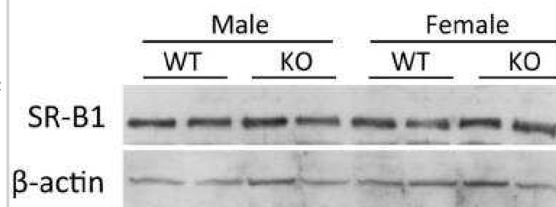
Flow (Intracellular): SR-BI Antibody [NB400-101] - An intracellular stain was performed on HeLa cells with NB400-101AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



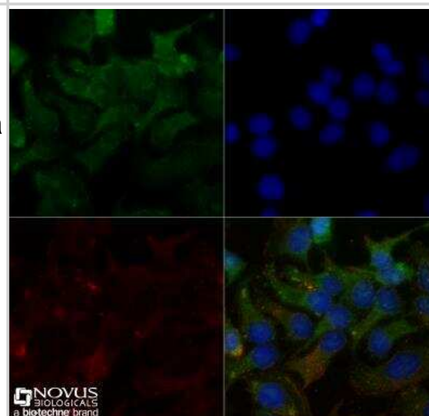
Knockout Validated: SR-BI Antibody [NB400-101] - Western blot and Coomassie stain of membrane fractions isolated from livers of normal C57Bl/6N (lane 1), Scarb1-KO (lane 2), and LIV11-SCARB1 x Scarb1-KO (lane 3) mice. 20 ug of membrane protein was loaded into each lane. (a)Western blot (anti-SR-BI antibody). (b) Coomassie stain (loading control for Western blot) encompassing the same MW region as SR-BI. Aliquots from the same tube were loaded for the Western blot and for the Coomassie-stained gel, which shows comparable loading between the three samples. Image collected and cropped by CiteAb from the following publication (http://www.hindawi.com/journals/bmri/2015/607120/) licensed under a CC-BY license.



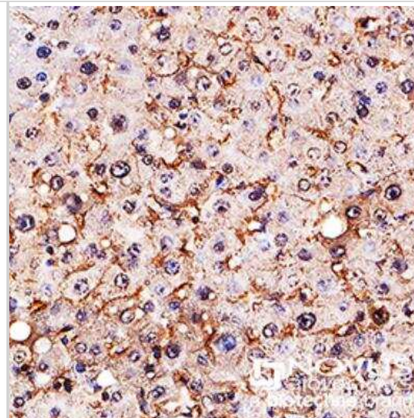
Western Blot: SR-BI Antibody [NB400-101] - Western blot analysis of ABCA1 and SR-B1 proteins from WT and Osbpl8KO mouse liver. The blots were probed with anti-beta-actin as a loading control. Densitometric quantification of the Western blot data is shown on the right. The results were normalized against beta-actin. The data represents mean +/- s.e.m. (n = 4). Image collected and cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0058856), licensed under a CC-BY license.



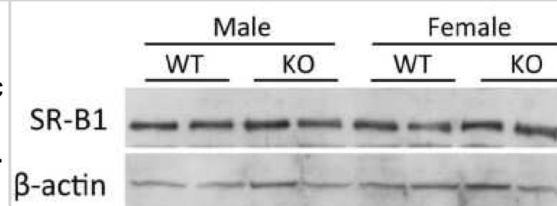
Immunocytochemistry/Immunofluorescence: SR-BI Antibody [NB400-101] - HeLa cells were fixed and permeabilized for 10 minutes using -20C MeOH. The cells were incubated with anti-SR-BI at 2 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



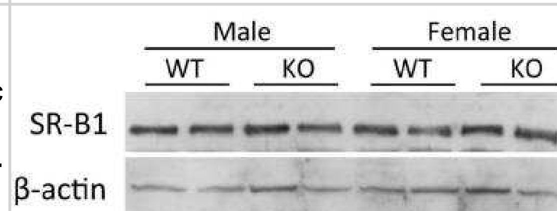
Immunohistochemistry-Paraffin: SR-BI Antibody [NB400-101] - SR-B1 was detected in immersion fixed paraffin-embedded sections of human liver using rabbit anti-human antibody (Catalog # NB400-101) at 1:300 dilution overnight at 4C. Tissue was stained using the VisuCyte anti-rabbit HRP polymer detection reagent (Catalog # VC003) with DAB chromogen (brown) and counterstained with hematoxylin (blue). Images may not be copied, printed or otherwise disseminated without express written permission of Novus Biologicals a Bio-techne brand.



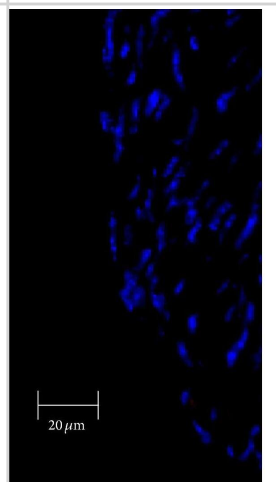
Western Blot: SR-BI Antibody [NB400-101] - Western blot analysis of ABCA1 and SR-B1 proteins from WT and Osbpl8KO mouse liver. The blots were probed with anti-beta-actin as a loading control. Densitometric quantification of the Western blot data is shown on the right. The results were normalized against beta-actin. The data represents mean +/- s.e.m. (n?=?4). Image collected and cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0058856), licensed under a CC-BY license.



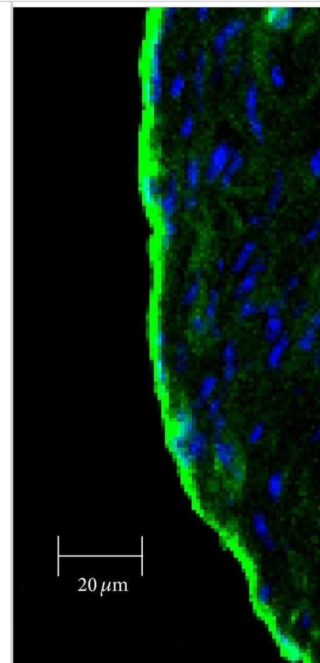
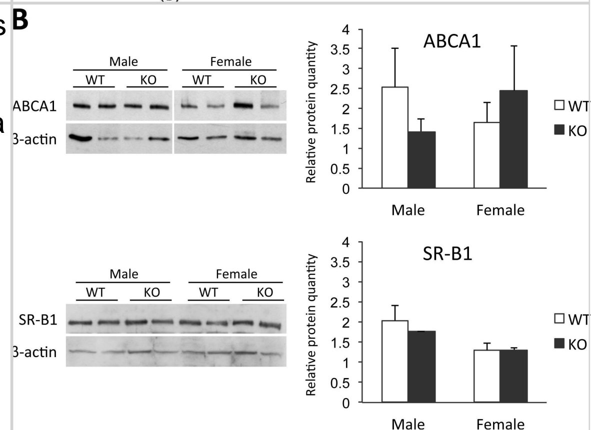
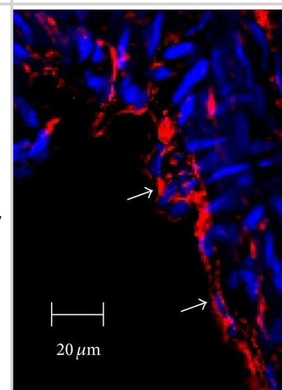
Western Blot: SR-BI Antibody [NB400-101] - Western blot analysis of ABCA1 and SR-B1 proteins from WT and Osbpl8KO mouse liver. The blots were probed with anti-beta-actin as a loading control. Densitometric quantification of the Western blot data is shown on the right. The results were normalized against beta-actin. The data represents mean +/- s.e.m. (n=4). Image collected and cropped by CiteAb from the following publication (http://dx.plos.org/10.1371/journal.pone.0058856), licensed under a CC-BY license.

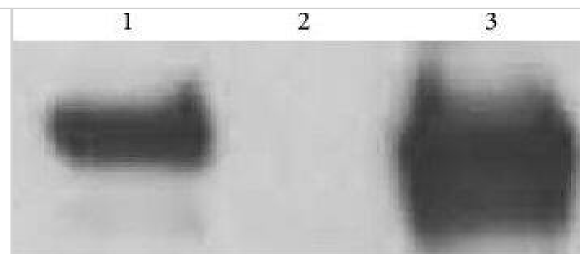
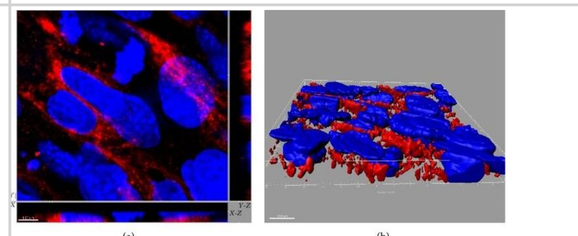
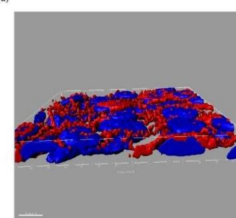
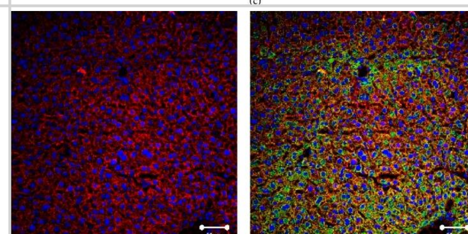
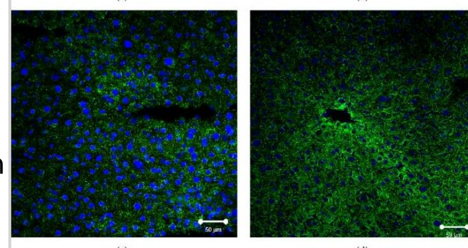


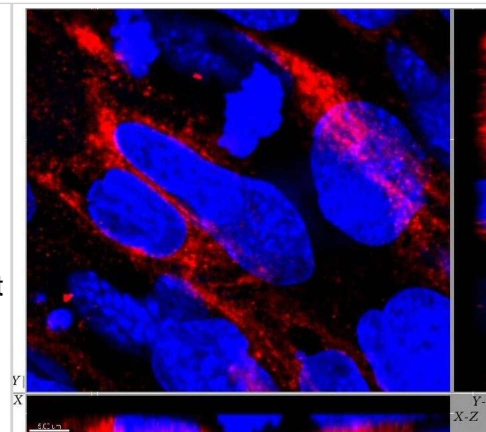
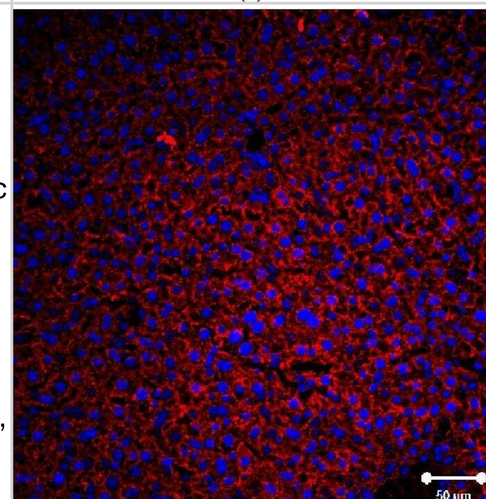
GDF11 inhibits TFEB activity. (a) AML12 cells were cultured for 48 h in presence of GDF11 (100 ng/ml). qRTPCR analysis of the mRNA levels of TFEB and its target genes VPS11 and ATP6V1H. (b) AML12 cells transient expressing GFPTFEB were cultured for 48 h in presence of GDF11 (100 ng/ml). Immunofluorescence confocal microscopy showing TFEB localization with or without GDF11 (original magnification, 400×). (c) Nuclear TFEB fluorescence intensity normalized to vehicle controls. (d) Western blot analysis of total TFEB protein expression. (e) Densitometry analysis of the total TFEB. (f) Western blot analysis of nuclear TFEB protein expression. (g) Densitometry analysis of the nuclear TFEB. The experiment was performed in triplicate with similar results. The data are shown as mean ± SD, *p < 0.05 compared to the vehicle group Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/34905649), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



(d)

Evidence that MRE11 and NBS1 are required for EBV episome maintenance.A) OriP plasmid maintenance assays were performed in MRE11 mutant (ATLD3) and reconstituted (ATLD3/wtMRE11) cell lines (left panel) or NBS1 mutant (GM7166) and reconstituted (GM7166/wtNBS1) cell lines (right panel). Plasmids containing OriP and EBNA1 were monitored by Southern blotting of Hirt lysates at 1 (lower panel) and 7 (upper panel) days post-transfection. Phosphorimager quantification of four independent experiments as shown in panal A where maintenance is measured as the ratio of day 7 to day 1 for OriP plasmid detection is shown below. B) EBV transformed B-lymphocytes were analyzed by PFG and Southern blotting for the presence of episomal forms of the EBV genome. Raji, Namalwa, NBS1 (GM15808), NBS1 (GM07078), LCL3472, and LCLAW7 DNA was loaded at equal concentrations and analyzed by ethidium staining of PFG (lower panel) or by Southern blot (upper panel) with EBV specific probe. C) Western blot of the same cell lines used for PFG analysis shown in panel B was probed with antibodies specific for NBS1 (antibody 143 and antibody 277), MRE11, or loading control Actin. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/18040525), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (f)
Initial characterization of the OS-9 protein.(A) OS-9 expression in various human cell lines. Equal protein amounts of total cell lysates were used for SDS-PAGE and subsequent Western blotting. For each cell line, two independent samples are shown. Endogenous OS-9 was detected with a polyclonal antibody raised against a peptide corresponding to amino acids 600–667 of isoform 1 of OS-9. (B) Protein stability assay of endogenous OS-9. U2OS cells were treated with the translational inhibitor cycloheximide (100 μM). At indicated time points, whole cell lysates were analysed by immunoblotting. (C) Effect of hypoxia on OS-9 expression. For hypoxia, UT-7 cells were exposed to 1% O2 for 24 h prior to Western blot analysis. To determine any influence of HIF-1α on OS-9 expression under normoxia, cells were incubated with the prolyl hydroxylase inhibitor DMOG (0.5 mM) for 24 h. (D) Protein interaction between OS-9 and PHD2 in vitro. For co-immunoprecipitation, U2OS cells were transiently co-transfected with the plasmids pOS-9-V5 and pPHD2-His, lysed in NP40 buffer, and subjected to immunoisolation with anti-V5 antibody recognizing OS-9 by its V5-tag. OS-9 and its associated proteins were separated by SDS-PAGE and analyzed by Western blot (lane 2). As controls, samples of untransfected (lane 1) cells or cells transfected with a single plasmid (lanes 3–4) were loaded. Representative Western blots are shown for each subfigure. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/21559462), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Murine tumor cells express connexin 43 (Cx43) and hypoxic-induced factor-1α (HIF-1α). The expression of Cx43 and HIF-1α was measured by Western blot analysis. β-actin expression served as loading controls and total protein. Inserted values indicated relative protein expression in comparison with β-actin. Image collected and cropped by CiteAb from the following open publication (http://www.mdpi.com/1422-0067/16/1/439), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (a)

Intracellular APE1/Ref-1 was targeted to the plasma membrane in response to acetylation. (A and B) Whole cell lysates were immunoprecipitated using an anti-acetyl lysine antibody, followed by immunoblotting with the polyclonal anti-APE1/Ref-1 antibody. The blots were stripped and re-probed with anti-β-actin and APE1/Ref-1 antibody to ensure equal protein loading. Similar results were obtained from replicate experiments. Column, mean (n = 3); bars, SE. *, $p < 0.05$, significantly different compared with control or between group by one-way ANOVA followed by Bonferroni's multiple comparison test. (C,D) Membrane fractions or whole cell lysates were prepared from the TSA-treated cells. Immunoblotting for APE1/Ref-1 was performed using the polyclonal anti-APE1/Ref-1 antibody. Blots were stripped and re-probed with anti-N-cadherin and anti-β-actin antibodies to control for differences in protein loading. Fold changes in the levels of APE1/Ref-1 in the plasma membrane fraction relative to the control are shown. †, indicates molecular marker (N-cadherin) of left image. Column mean (n = 3); bars, SE.*, $p < 0.05$ indicates a significantly different result compared with control or between groups by one-way ANOVA followed by Bonferroni's multiple comparison test. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/31261750), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (a)
Hif1α expression in neonatal and adult testis.(A) Section of testis from 5-day old (P5) male new born pups showing Hif1α expression in MVH+ gonocytes within the seminiferous tubules (top) and negative control images without primary antibodies (bottom). Scale bar: 50μm. (B) Western blot analysis of Hif1α expression in P5 testes (left) compared to extract of the adult brain sub-ventricular zone (SVZ) (right). Loading control (β-Actin) is shown below. (C) Section of adult (3 month old) testis showing Hif1α expression in spermatogonia. Scale bar: 30μm. (D) Western blot analysis of whole adult testis. HEK293 cells treated with DFX were used as a positive control and intestinal tissue was used as a negative control. Loading control (β-Actin) is shown below. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/27148974), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (a) (b)  (c)
UDM1 is required for RNF168-mediated 53BP1 recruitment.a Schematic illustration of RNF8 and RNF168 structural domain organization, RNF168 fragments, and chimeric proteins used as in b and c. FHA–forkhead-association domain; coiled-coil–coiled-coil domain; RING–ubiquitin E3 ligase RING domain; Rs-arginine anchor; LRM–LR motif; UMI–UIM-and MIU-related ubiquitin binding domain; MIU–motif interacting with ubiquitin. b–c Transient transfection of GFP-tagged RNF168 fragments and chimeric proteins in U2OS RNF168 KO cells. Cells were irradiated with 10 Gy and allowed to recover for 1 h followed by immunofluorescence analysis using 53BP1 antibody as indicated. Cells were counterstained with DAPI. Repeated at least two times independently with similar results. Source data are provided as Source Data file. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32424115), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (a) (b)  (c) (d)

Vascularization of an engrafted patient colon tumor. A patient colon tumor was implanted in a cohort of mice and vessel development was analyzed over 8 weeks. Vessels in the original patient specimen labeled strongly for huCD31 (A) and not for msCD34 (D). Representative sections showing loss of huCD31(+) vessels (B- 4 weeks, C- 7 weeks) and presence of msCD34(+) vessels (E- 4 weeks, F- 7 weeks) are shown. The graph (G) summarizes this process; huCD31(+) vessels were rapidly lost, and by one week, mouse vessels were the predominate vessels present in the colon tumors (no data for msCD34 at 2 weeks; bars = 100 μ). Image collected and cropped by CiteAb from the following open publication (https://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-11-110), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (a)
The DNA damage response at telomeres uncapped through TRF2 depletion does not require BRCA1 or CtIPImmortalized Brca1F/− MEFs were infected with retroviruses expressing the indicated shRNAs and/or Cre recombinase, followed by selection with puromycin for 72 h. Cell extracts were prepared 48 h later and analysed by Western blotting as indicated. SMC1 and tubulin were used as loading controls. *non-specific band.Cells treated as in (A) were fixed 48 h after selection and stained with an anti-53BP1 antibody (green). Telomeres were visualized with a Cy3-conjugated (CCCTAA)3-PNA probe (red). Yellow arrowheads point to 53BP1 foci that co-localize with telomeres.Quantification of TIFs in cells treated as in (B). A minimum of 200 nuclei were scored for each sample. Error bars represent SD of two independent experiments. P-values were calculated using an unpaired two-tailed t-test. *P ≤ 0.05; NS, P > 0.05. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25582120), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (a)

Publications

Xu H, Thomas MJ, Kaul S et al. Pcpe2, a Novel Extracellular Matrix Protein, Regulates Adipocyte SR-BI-Mediated High-Density Lipoprotein Uptake Arteriosclerosis, Thrombosis, and Vascular Biology 2021-11-01 [PMID: 34551590] (B/N, WB)

Xu Y, Zhu Y, Hu S et al. Hepatocyte Nuclear Factor 4? Prevents the Steatosis-to-NASH Progression by Regulating p53 and Bile Acid Signaling (in mice) Hepatology 2021-06-01 [PMID: 33098092] (WB)

Toutonji A, Krieg C, Borucki DM et al. Mass cytometric analysis of the immune cell landscape after traumatic brain injury elucidates the role of complement and complement receptors in neurologic outcomes Acta neuropathologica communications 2023-06-12 [PMID: 37308987] (CyTof, Mouse)

Li Z, He M, Chen G et al. Effect of Total Sphingomyelin Synthase Activity on Low Density Lipoprotein Catabolism in Mice bioRxiv : the preprint server for biology 2023-02-07 [PMID: 36798262] (WB, Mouse)

May SC, Sahoo D A short amphipathic alpha helix in scavenger receptor BI facilitates bidirectional HDL-cholesterol transport The Journal of biological chemistry 2022-08-01 [PMID: 35926711] (WB, Human)

Details:
WB 1:5000

Anthony H, Thomas O, Martin C et al. Cholesterol supports bovine granulosa cell inflammatory responses to lipopolysaccharide Society for Reproduction and Fertility 2022-08-05 [PMID: 35900358] (WB, Bovine)

Hofmann M. Influence of the Maillard reaction on the allergenicity of food allergens Curr Allergy Asthma Rep 2019-01-29 [PMID: 30689122]

Zheng KH, Kroon J, Schoormans J et al. 89Zr-labeled High-Density Lipoprotein Nanoparticle PET imaging reveals tumor uptake in patients with esophageal cancer Journal of nuclear medicine : official publication, Society of Nuclear Medicine 2022-06-23 [PMID: 35738904]

Shinohata R, Shibakura M, Arao Y et al. A high-fat/high-cholesterol diet, but not high-cholesterol alone, increases free cholesterol and apoE-rich HDL serum levels in rats and upregulates hepatic ABCA1 expression Biochimie 2022-01-24 [PMID: 35085709] (WB, Rat)

Dalton GD, Oh SH, Tang L et al. Hepatocyte activity of the cholesterol sensor smoothened regulates cholesterol and bile acid homeostasis in mice iScience 2021-09-24 [PMID: 34568800] (WB, Mouse)

Baranova IN, Bocharov AV, Vishnyakova TG et al. Class B Scavenger Receptors BI and BII Protect Against LPS-induced Acute Lung Injury in Mice by Mediating LPS Clearance Infection and immunity 2021-06-07 [PMID: 34097506]

Bouillet B, Gautier T, Denimal D Et Al. Glucocorticoids impair HDL-mediated cholesterol efflux besides increased HDL cholesterol concentration - a proof of concept Eur. J. Endocrinol. 2020-06-01 [PMID: 32570209] (WB)

More publications at http://www.novusbio.com/NB400-101

Procedures

Western Blot protocol for SR-BI Antibody (NB400-101)
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.

Immunohistochemistry-Paraffin Protocol for SR-BI Antibody (NB400-101)
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.

Immunocytochemistry/ Immunofluorescence Protocol for SR-BI Antibody (NB400-101)
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.

Flow (Intracellular) Protocol for SR-BI Antibody (NB400-101)
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 permeabilization 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 permeabilization 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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Products Related to NB400-101

NB820-59662	Mouse Liver Whole Tissue Lysate (Adult Whole Normal)
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NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
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