Product Datasheet

SR-BI Antibody - BSA Free

NB400-101

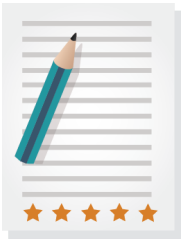
Unit Size: 0.1 ml

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

Reviews: 1 Publications: 114

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB400-101

Updated 2/17/2025 v.20.1



NB400-101

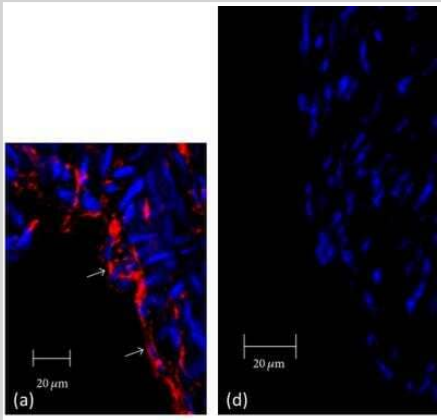
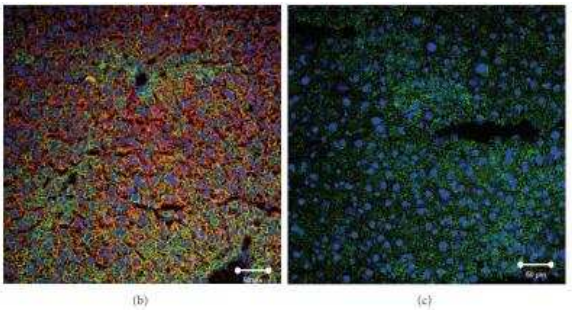
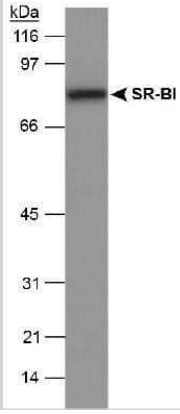
SR-BI Antibody - BSA Free

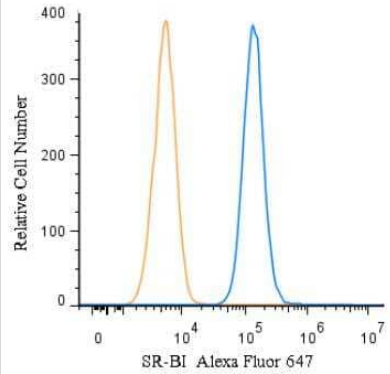
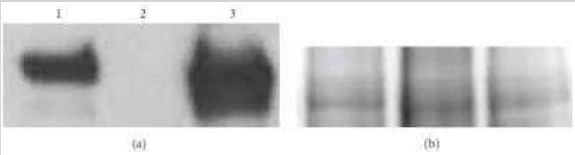
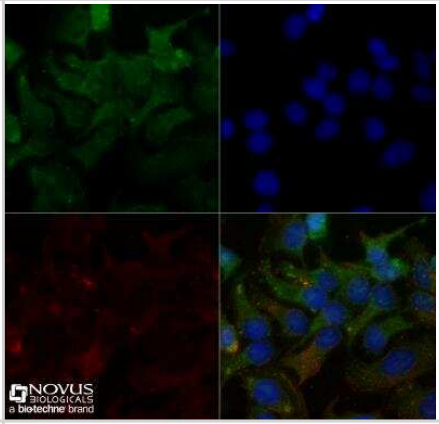
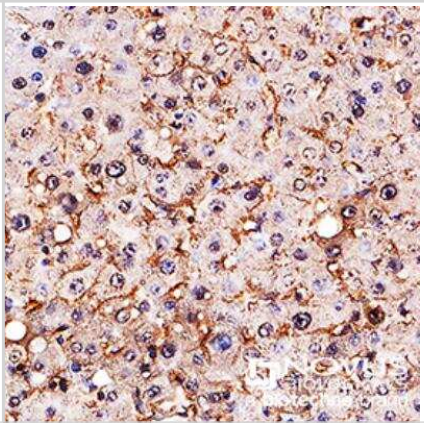
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	82 kDa

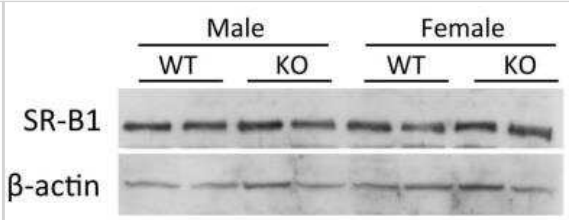
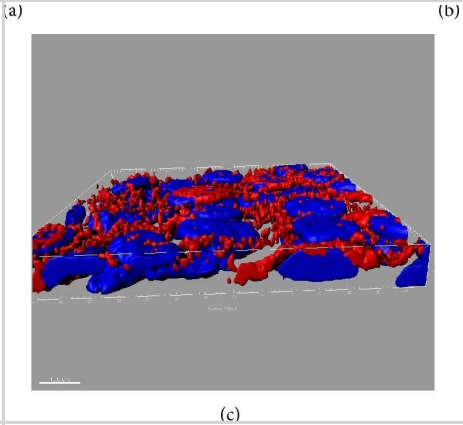
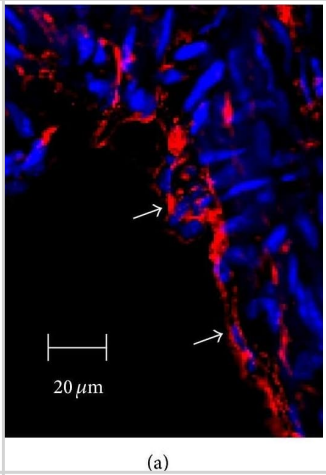
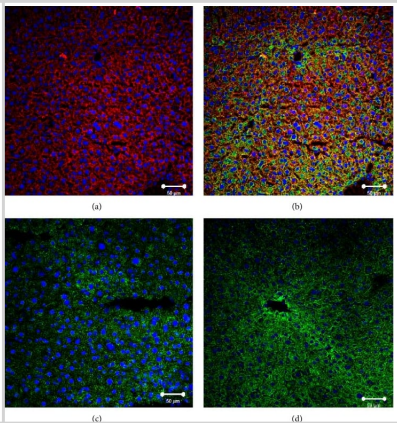
Product Description	
Host	Rabbit
Gene ID	949
Gene Symbol	SCARB1
Species	Human, Mouse, Rat, Bovine, Chinese Hamster, Mustelid, Primate, S. japonicum
Reactivity Notes	Bovine reactivity reported in scientific literature (PMID: 24196350).
Immunogen	A C-terminal peptide containing residues from mouse SR-BI (within residues 450 -509). [Uniprot: Q61009]

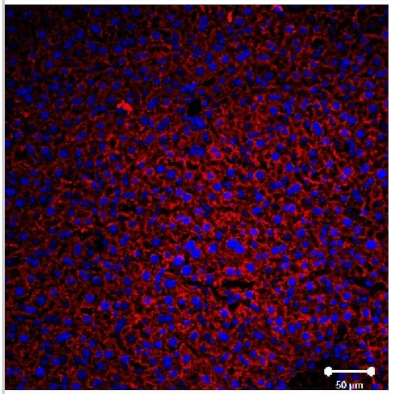
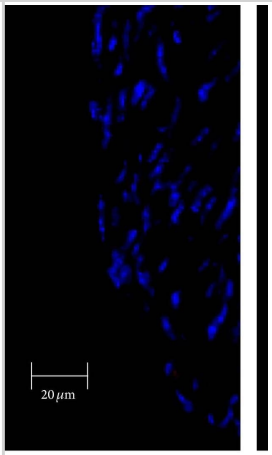
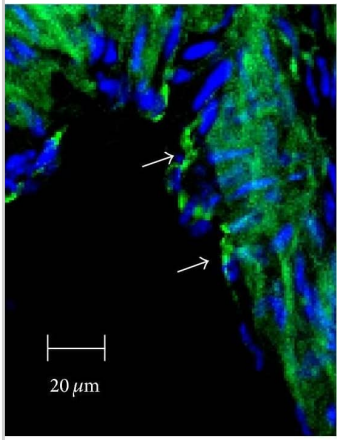
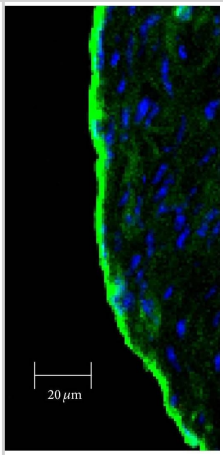
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Flow (Intracellular), Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Block/Neutralize, Knockout Validated
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. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100, apparent MW was 74 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

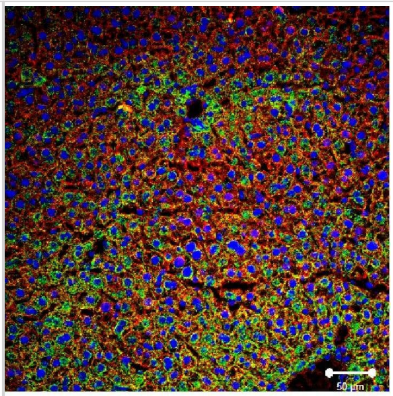
Immunocytochemistry/ Immunofluorescence: SR-BI Antibody [NB400-101] - Immunofluorescent analysis of SR-BI localization in aorta of Tie2-Scarb1 x Scarb1-KO and Scarb1-KO mice. Mouse aorta sections were stained for SR-BI (a and d, red). In several EC stained for SR-B1 red color was present on both apical 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.	
Immunohistochemistry: 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 (https://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.	
Western Blot: 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 (https://www.hindawi.com/journals/bmri/2015/607120/) 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 (https://dx.plos.org/10.1371/journal.pone.0058856), licensed under a CC-BY license.	
Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - SR-BI distribution in polarized EC SVEC4-10 cells. SR-BI localization is presented in (a) as x-y image & orthogonal (x-z & y-z) views. (b) & (c) represent rendering of SR-BI staining (red) throughout the cells. Nuclei: blue. In (b) & (c) basolateral surface is on the top & the bottom, respectively. These results demonstrate the presence of SR-BI on both the apical & the basolateral sides of EC. The size of scale bar on (a) is 5 µm & on (b) & (c) 10 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - Immunofluorescent analysis of SR-BI localization in aorta of Tie2-Scarb1 × Scarb1-KO & Scarb1-KO mice. Mouse aorta sections were stained for SR-BI (a & d, red) & CD31 (b & e, green). Colocalization of SR-BI & CD31 was presented on (c) & (f). Blue = DAPI. (a, b, & c) Immunohistological analysis of SR-BI localization in aortic sections of Tie2-Scarb1 × Scarb1-KO mice. Colocalization of SR-BI & CD31 was found in aortic endothelial cells. In several EC stained for SR-B1 red color was present on both apical & basolateral sides (a). (d, e, & f) Immunohistological analysis of SR-BI localization in aortic sections of Scarb1-KO mice. There is no red signal in aorta from Scarb1-KO mice. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - Immunofluorescent analysis of SR-BI localization in liver of normal, Tie2-Scarb1 × Scarb1-KO, & Scarb1-KO mice. Liver sections from normal (a, b) & Tie2-Scarb1 × Scarb1-KO mice (c) were stained for SR-BI (red) & cytokeratin 8–18 (green). Merge image for normal mouse (b) demonstrates strong presence of SR-BI in hepatocytes (b, yellow signal) & absence of detectable level of SR-BI protein in liver of Tie2-Scarb1 × 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 & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

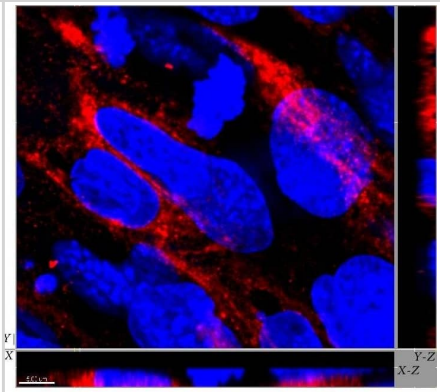
Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - Immunofluorescent analysis of SR-BI localization in liver of normal, Tie2-Scarb1 × Scarb1-KO, & Scarb1-KO mice. Liver sections from normal (a, b) & Tie2-Scarb1 × Scarb1-KO mice (c) were stained for SR-BI (red) & cytokeratin 8–18 (green). Merge image for normal mouse (b) demonstrates strong presence of SR-BI in hepatocytes (b, yellow signal) & absence of detectable level of SR-BI protein in liver of Tie2-Scarb1 × 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 & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (a)
Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - Immunofluorescent analysis of SR-BI localization in aorta of Tie2-Scarb1 × Scarb1-KO & Scarb1-KO mice. Mouse aorta sections were stained for SR-BI (a & d, red) & CD31 (b & e, green). Colocalization of SR-BI & CD31 was presented on (c) & (f). Blue = DAPI. (a, b, & c) Immunohistological analysis of SR-BI localization in aortic sections of Tie2-Scarb1 × Scarb1-KO mice. Colocalization of SR-BI & CD31 was found in aortic endothelial cells. In several EC stained for SR-B1 red color was present on both apical & basolateral sides (a). (d, e, & f) Immunohistological analysis of SR-BI localization in aortic sections of Scarb1-KO mice. There is no red signal in aorta from Scarb1-KO mice. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (d)
Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - Immunofluorescent analysis of SR-BI localization in aorta of Tie2-Scarb1 × Scarb1-KO & Scarb1-KO mice. Mouse aorta sections were stained for SR-BI (a & d, red) & CD31 (b & e, green). Colocalization of SR-BI & CD31 was presented on (c) & (f). Blue = DAPI. (a, b, & c) Immunohistological analysis of SR-BI localization in aortic sections of Tie2-Scarb1 × Scarb1-KO mice. Colocalization of SR-BI & CD31 was found in aortic endothelial cells. In several EC stained for SR-B1 red color was present on both apical & basolateral sides (a). (d, e, & f) Immunohistological analysis of SR-BI localization in aortic sections of Scarb1-KO mice. There is no red signal in aorta from Scarb1-KO mice. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (b)
Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - Immunofluorescent analysis of SR-BI localization in aorta of Tie2-Scarb1 × Scarb1-KO & Scarb1-KO mice. Mouse aorta sections were stained for SR-BI (a & d, red) & CD31 (b & e, green). Colocalization of SR-BI & CD31 was presented on (c) & (f). Blue = DAPI. (a, b, & c) Immunohistological analysis of SR-BI localization in aortic sections of Tie2-Scarb1 × Scarb1-KO mice. Colocalization of SR-BI & CD31 was found in aortic endothelial cells. In several EC stained for SR-B1 red color was present on both apical & basolateral sides (a). (d, e, & f) Immunohistological analysis of SR-BI localization in aortic sections of Scarb1-KO mice. There is no red signal in aorta from Scarb1-KO mice. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (f)

Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - Immunofluorescent analysis of SR-BI localization in liver of normal, Tie2-Scarb1 × Scarb1-KO, & Scarb1-KO mice. Liver sections from normal (a, b) & Tie2-Scarb1 × Scarb1-KO mice (c) were stained for SR-BI (red) & cytokeratin 8–18 (green). Merge image for normal mouse (b) demonstrates strong presence of SR-BI in hepatocytes (b, yellow signal) & absence of detectable level of SR-BI protein in liver of Tie2-Scarb1 × 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 & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



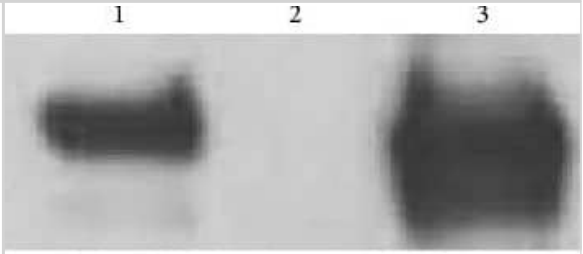
(b)

Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - SR-BI distribution in polarized EC SVEC4-10 cells. SR-BI localization is presented in (a) as x-y image & orthogonal (x-z & y-z) views. (b) & (c) represent rendering of SR-BI staining (red) throughout the cells. Nuclei: blue. In (b) & (c) basolateral surface is on the top & the bottom, respectively. These results demonstrate the presence of SR-BI on both the apical & the basolateral sides of EC. The size of scale bar on (a) is 5 µm & on (b) & (c) 10 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



(a)

Western Blot: SR-BI Antibody - BSA Free [NB400-101] - Western blot & Coomassie stain of membrane fractions isolated from livers of normal C57Bl/6N (lane 1), Scarb1-KO (lane 2), & LIV11-SCARB1 × Scarb1-KO (lane 3) mice. 20 µg 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 & for the Coomassie-stained gel, which shows comparable loading between the three samples. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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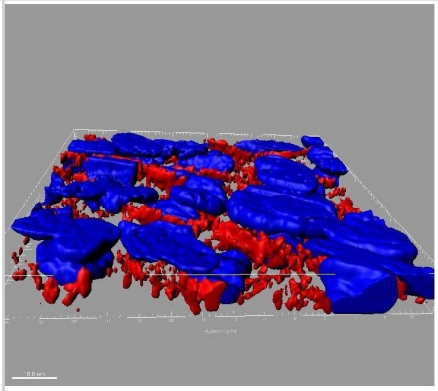

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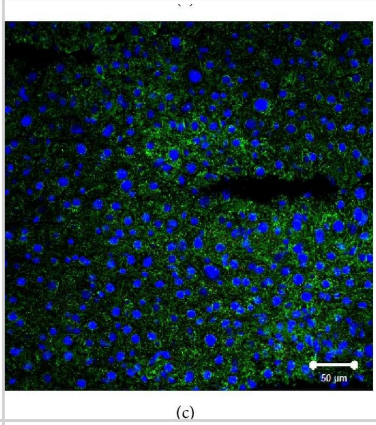
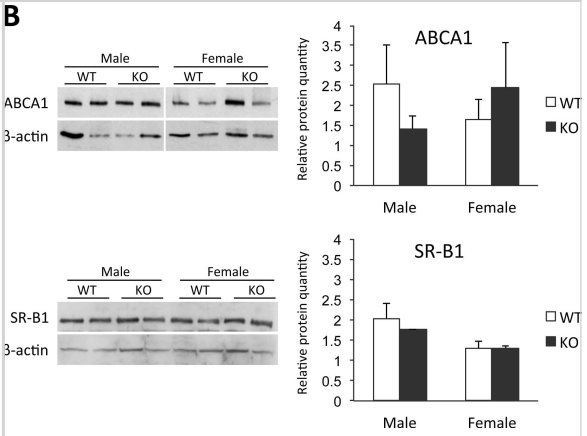
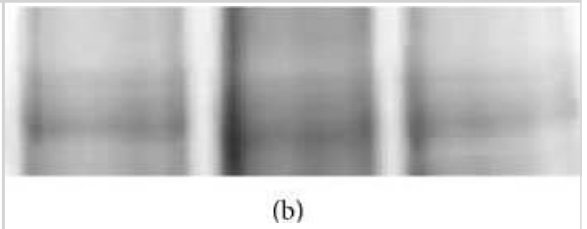


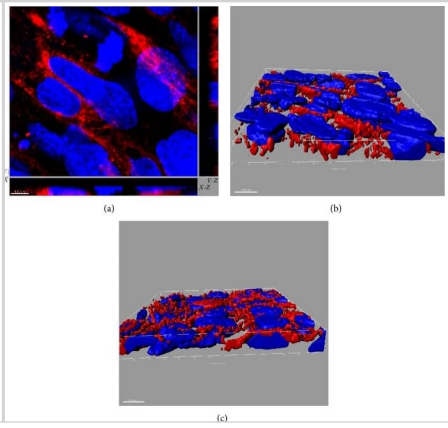
Western Blot: SR-BI Antibody - BSA Free [NB400-101] - Liver mRNA & protein expression analysis of chow-fed Osbpl8KO mice.A: qPCR analysis of the quantity of the mRNAs identified at the bottom in chow-fed KO females (open bars) & males (closed bars). The mRNAs were quantified using ribosomal protein 36B4 message as a housekeeping reference. The data are expressed relative to quantity in littermate WT animals of the same gender, & represent mean ± s.e.m. (n=6; *p<0.05, **p<0.01, T-test). B: Western blot analysis of ABCA1 & SR-B1 proteins in WT & KO mouse liver. The blots were probed with anti-β-actin as a loading control. Densitometric quantification of the Western blot data is shown on the right. The results were normalized against β-actin. The data represents mean ± s.e.m. (n=4). Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0058856), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

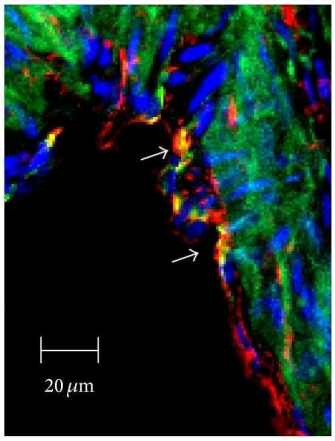
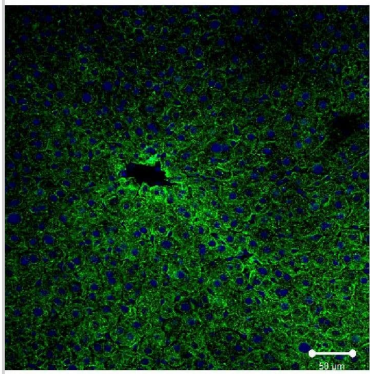


Western Blot: SR-BI Antibody - BSA Free [NB400-101] - Western blot & Coomassie stain of membrane fractions isolated from livers of normal C57Bl/6N (lane 1), Scarb1-KO (lane 2), & LIV11-SCARB1 × Scarb1-KO (lane 3) mice. 20 µg of membrane protein was loaded into each lane. (a) Western blot (anti-SR-BI 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 & for the Coomassie-stained gel, which shows comparable loading between the three samples. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - SR-BI distribution in polarized EC SVEC4-10 cells. SR-BI localization is presented in (a) as x-y image & orthogonal (x-z & y-z) views. (b) & (c) represent rendering of SR-BI staining (red) throughout the cells. Nuclei: blue. In (b) & (c) basolateral surface is on the top & the bottom, respectively. These results demonstrate the presence of SR-BI on both the apical & the basolateral sides of EC. The size of scale bar on (a) is 5 µm & on (b) & (c) 10 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - Immunofluorescent analysis of SR-BI localization in aorta of Tie2-Scarb1 × Scarb1-KO & Scarb1-KO mice. Mouse aorta sections were stained for SR-BI (a & d, red) & CD31 (b & e, green). Colocalization of SR-BI & CD31 was presented on (c) & (f). Blue = DAPI. (a, b, & c) Immunohistological analysis of SR-BI localization in aortic sections of Tie2-Scarb1 × Scarb1-KO mice. Colocalization of SR-BI & CD31 was found in aortic endothelial cells. In several EC stained for SR-B1 red color was present on both apical & basolateral sides (a). (d, e, & f) Immunohistological analysis of SR-BI localization in aortic sections of Scarb1-KO mice. There is no red signal in aorta from Scarb1-KO mice. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (c)
Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-101] - Immunofluorescent analysis of SR-BI localization in liver of normal, Tie2-Scarb1 × Scarb1-KO, & Scarb1-KO mice. Liver sections from normal (a, b) & Tie2-Scarb1 × Scarb1-KO mice (c) were stained for SR-BI (red) & cytokeratin 8–18 (green). Merge image for normal mouse (b) demonstrates strong presence of SR-BI in hepatocytes (b, yellow signal) & absence of detectable level of SR-BI protein in liver of Tie2-Scarb1 × 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 & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26504816), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	 (d)

Publications

Pan X, Hu S, Xu Y et Al. Krüppel-like factor 10 protects against metabolic dysfunction-associated steatohepatitis by regulating HNF4?-mediated metabolic pathways Metabolism 2024-06-07 [PMID: 38582490]

Liu Z, Ruter DL, Quigley K et Al. Single-Cell RNA Sequencing Reveals Endothelial Cell Transcriptome Heterogeneity Under Homeostatic Laminar Flow Arterioscler Thromb Vasc Biol 2021-08-26 [PMID: 34433297] (Western Blot)

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Srividya Velagapudi, Dongdong Wang, Francesco Poti, Renata Feuerborn, Jerome Robert, Eveline Schlumpf, Mustafa Yalcinkaya, Grigorios Panteloglou, Anton Potapenko, Manuela Simoni, Lucia Rohrer, Jerzy-Roch Nofer, Arnold von Eckardstein Sphingosine-1-phosphate receptor 3 regulates the transendothelial transport of high-density lipoproteins and low-density lipoproteins in opposite ways Cardiovascular Research 2024-04-01 [PMID: 38109696]

B Fuenzalida, C Cantin, S Kallol, L Carvajal, V Pastén, S Contreras-, C Albrecht, J Gutierrez, A Leiva Cholesterol uptake and efflux are impaired in human trophoblast cells from pregnancies with maternal supraphysiological hypercholesterolemia Sci Rep, 2020-03-24;10(1):5264. 2020-03-24 [PMID: 32210256]

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] (Western Blot, Block/Neutralize)

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] (Western Blot)

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]

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)
NB400-101PEP	SR-BI 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.

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