Product Datasheet

SR-BI Antibody
NB400-104

Unit Size: 0.1 ml
Store at 4C. Do not freeze.

Reviews: 4  Publications: 163

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Updated 8/22/2018 v.20.1

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### Product Information

<table>
<thead>
<tr>
<th><strong>Unit Size</strong></th>
<th>0.1 ml</th>
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<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Store at 4C. Do not freeze.</td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
<td>Polyclonal</td>
</tr>
<tr>
<td><strong>Preservative</strong></td>
<td>0.1% Sodium Azide</td>
</tr>
<tr>
<td><strong>Isotype</strong></td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Purity</strong></td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td>PBS</td>
</tr>
<tr>
<td><strong>Target Molecular Weight</strong></td>
<td>82 kDa</td>
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### Product Description

<table>
<thead>
<tr>
<th><strong>Host</strong></th>
<th>Rabbit</th>
</tr>
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<tbody>
<tr>
<td><strong>Gene ID</strong></td>
<td>949</td>
</tr>
<tr>
<td><strong>Gene Symbol</strong></td>
<td>SCARB1</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>Human, Mouse, Rat, Chinese Hamster, Hamster, Mustelid, Primate, Rabbit, Golden Syrian Hamster</td>
</tr>
<tr>
<td><strong>Reactivity Notes</strong></td>
<td>Mouse, rat, mink, hamster, primate, rabbit and the long form of human SR-BI. Reactivity with CHO cells reported in scientific literature (PMID: 12356718).</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>A C-terminal peptide containing residues from mouse SR-BI (within residues 450-509). [UniProt# Q61009]</td>
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### Product Application Details

<table>
<thead>
<tr>
<th><strong>Applications</strong></th>
<th>Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended Dilutions</strong></td>
<td>Western Blot 1:1000-1:5000, Simple Western 1:100, Flow Cytometry 1:10 - 1:1000, 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</td>
</tr>
<tr>
<td><strong>Application Notes</strong></td>
<td>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. Use in Immunohistochemistry-Frozen reported in scientific literature (PMID 26865459). In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.</td>
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Images

Simple Western: SR-BI Antibody [NB400-104] - Simple Western 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.

Western Blot: SR-BI Antibody [NB400-104] - Analysis using the HRP conjugate of NB400-104. Detection of SR-BI in 25 ug of mouse liver lysate, using NB400-104 (lotJ) at a 1:1,000 dilution, shows distinct band at 82 kDa.


Immunocytochemistry/Immunofluorescence: SR-BI Antibody [NB400-104] - SR-BI antibody was tested in human fibroblast samples fixed in 4% PFA and permeabilized in PBS (0.2% Tween). Primary incubation was performed overnight at 4C using a 1:100 dilution in PBS (0.1% Tween) with 1% bovine serum albumin. Secondary antibody is anti-rabbit conjugate to Alexa Fluor 488. SR-BI is shown in green and nucleus in blue (Hoescht 33342 stain).
Flow Cytometry: SR-BI Antibody [NB400-104] - An intracellular stain was performed on HeLa cells with SR-BI antibody NB400-104AF488 (blue) and a matched isotype control NBP2-24893AF488 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 µg/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.

Immunohistochemistry-Paraffin: SR-BI Antibody [NB400-104] - IHC analysis of a formalin fixed paraffin embedded tissue section of Mouse liver using SR-BI antibody (Lot 8310) at 1:300 dilution with HRP-DAB detection and hematoxylin counterstaining. The antibody generated a specific membrane signal of SR-BI protein in the murine hepatocytes.


Flow Cytometry: SR-BI Antibody [NB400-104] - An intracellular stain was performed on A549 cells with SR-BI antibody NB400-104AF488 (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 µg/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.
Western Blot: SR-BI Antibody [NB400-104] - SR-BI antibody was tested in human adrenal cell lysate.

Immunocytochemistry/Immunofluorescence: SR-BI Antibody [NB400-104] - 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: SR-BI Antibody [NB400-104] - Immunolocalization of SR-BI in adult mink testis using NB400-104. SR-BI labeling is visible at the surface and along the outline of the large vacuole. Photo courtesy of R.M. Pelletier, University of Montreal.

Immunohistochemistry-Paraffin: SR-BI Antibody [NB400-104] - IHC analysis of a formalin fixed paraffin embedded tissue section of Mouse liver using SR-BI antibody (Lot R-4) at 1:300 dilution with HRP-DAB detection and hematoxylin counterstaining. The antibody generated mainly a membranous signal of SR-BI protein in the murine hepatocytes.

Flow Cytometry: SR-BI Antibody [NB400-104] - An intracellular stain was performed on HeLa cells with SR-BI antibody NB400-104PE (blue) and a matched isotype control NBP2-24893PE (orange). Cells were fixed with 4% PFA and then permeabized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.

Flow Cytometry: SR-BI Antibody [NB400-104] - SR-B1 staining of human adipose tissue macrophage populations compared to isotype control (orange). Image from verified customer review. Image using the DyLight 550 form of this antibody.
<table>
<thead>
<tr>
<th>Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Durham KK, Chathely KM, Trigatti BL. High density lipoprotein protects cardiomyocytes against necrosis induced by oxygen and glucose deprivation, through SR-B1, PI3K, and AKT1 and 2. Biochem. J. Mar 9 2018 12:00AM [PMID: 29523748] (Mouse)</td>
</tr>
<tr>
<td>Zhu L, Shi J, Luu TN et al. Hepatocyte estrogen receptor alpha mediates estrogen action to promote reverse cholesterol transport during Western-type diet feeding. Molecular Metabolism 2017 [PMID: 29331506] (Mouse)</td>
</tr>
<tr>
<td>Details:</td>
</tr>
<tr>
<td>This citation used the Alexa Fluor 647 form of this antibody.</td>
</tr>
<tr>
<td>More publications at <a href="http://www.novusbio.com/NB400-104">http://www.novusbio.com/NB400-104</a></td>
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Procedures

Western Blot Protocol for SR-BI Antibody (NB400-104)
Procedure Guide for NB 400-104 Polyclonal anti-SR-B1

Western Blot Procedure
1. Run ~50 ug of protein on a 4-20% Tris-glycine mini-gel at 125V for 60 minutes.
2. Equilibrate gel, nitrocellulose membrane, Whatman paper, and blotting pads in transfer buffer for 15 minutes.
3. Transfer protein to the membrane at 25V for 90 minutes.
4. Allow membrane to air-dry.
5. Block membrane with 1XPBS/5% non-fat milk/0.1% Tween-20 for 1 hour at room temperature (~23-27 degrees C).
6. Wash membrane twice, for 5 minutes each, with 1XPBS/0.05% Tween-20 (PBST).
7. Incubate membrane with 1:250 dilution of NB400-104 (anti-SR-BI), diluted in 1XPBS/1% BSA, for 1 hour at room temperature.
8. Wash membrane once for 15 minutes, then four times for 5 minutes each, with PBST.
9. Incubate membrane with 1:10,000 dilution of goat anti-rabbit IgG-HRP (BioRad), diluted in 1XPBS/1% BSA, for 1 hour at room temperature.
10. Wash membrane once for 15 minutes, then four times for 5 minutes each, with PBST.
11. Detect cross-reacting proteins using Renaissance Chemiluminescence Reagent Plus kit from NEN Life Sciences. NOTE: HL-60 whole cell extracts (NB800-PC3) were used as a positive control for this antibody.

FACS Procedure
1. Resuspend 1x10^6 cells in FACS Buffer.
2. Pellet at 1600 rpm for 48 seconds; in a 96-well plate format
3. Resuspend in 50 uL FACS Buffer containing 1:100 dilution of SR-B1 Ab (NB 400-104) or mouse Isotype control Ab
4. Incubate at 4 degrees C for 1 hour
5. Pellet at 1600 rpm for 48 seconds
6. Wash three times with 150 ul FACS buffer
7. Resuspend in 50 uL FACS Buffer containing 1:200 dilution of anti-mouse IgG-PE Ab (Becton Dickinson Cat# 550083)
8. Incubate at 4 degrees C for 1 hour
9. Pellet at 1600 rpm for 48 seconds
10. Wash three times with 150 ul FACS buffer
11. Resuspend in 150 ul FACS Buffer for analysis on a Becton Dickinson FACSCalibur.

IHC-FFPE sections

I. Deparaffinization:
A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:
A. Place slides in peroxidase quenching solution: 15-30 minutes.
To Prepare 200 ml of Quenching Solution:
3. Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
Use within 4 hours of preparation
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:
A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.

D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution.
Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (<em>do not wash off the Blocking Reagent</em>), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes.
Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.

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technical@novusbio.com
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:
- Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
- Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.
- All steps in which Xylene is used should be performed in a fume hood.
- For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
- For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
- 200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
- 5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
- Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).
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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