# **Product Datasheet**

# SR-BI Antibody - BSA Free NB400-104

Unit Size: 0.1 ml

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

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# NB400-104

SR-BI Antibody - BSA Free

Product Information		
Unit Size	0.1 ml	
Concentration	1.0 mg/ml	
Storage	Store at 4C short term. 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, Porcine, Chinese Hamster, Hamster, Mustelid, Primate, Rabbit, Golden Syrian Hamster	
Reactivity Notes	Use in Mouse reported in scientific literature (PMID:33719499).	
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), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Proximity Ligation Assay, Block/Neutralize, Knockdown Validated, Knockout Validated	
Recommended Dilutions	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 reported in scientific literature (PMID 26865459), Proximity Ligation Assay reported in scientific literature (10.1016/j.jbc.2021.100828), Flow (Intracellular), Knockout Validated reported in scientific literature (PMID 31462534), Knockdown Validated, Block/Neutralize reported in scientific literature (PMID 24859737)	
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 <u>Simple Western Antibody Database</u> for Simple Western validation: Tested in HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100, apparent MW was 78 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.	



Images	
Simple Western: SR-BI Antibody [NB400-104] - Image 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.	KDy 230- 180- 116
Western Blot: SR-BI Antibody [NB400-104] - SR-BI antibody was tested in human adrenal cell lysate.	<250 <150 <100 <75 <50 <37 <25 <20 <15 <10
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 overnight at 4C using a 1:100 dilution in PBS (0.1% Tween) with 1% BSA. 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 permeablized 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.	$300 \frac{1}{100} $



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Immunohistochemistry-Paraffin: SR-BI Antibody [NB400-104] - Staining of human adrenal cortex.

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

1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain

Immunohistochemistry-Paraffin: SR-BI Antibody [NB400-104] - FFPE 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

imaged using a 40X objective.

murine hepatocytes.



overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 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









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C57BL/6J

+ vehicle



CD68 positive KCs. Antibodies to stabilin-2 & FcγRIIb2 (SE-1) specifically labeled LSECs & the CD68-antibody specifically labeled KCs, whereas positive labeling for the mannose receptor, SR-A1, & SR-B1 was observed in both LSECs & KCs Image collected & cropped by CiteAb from the following publication

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Western Blot: SR-BI Antibody - BSA Free [NB400-104] - Protein expressions of ABCA1, SR-BI & ABCG1 in liver & macrophage by Western blot. Simvastatin increased the expressions of ABCA1 & ABCG1 in liver & ABCA1 in macrophage, Both L-4F & the combination group improved the expressions of ABCA1, SR-BI & ABCG1 in liver & ABCA1 & ABCG1 in macrophage. 1P < 0.05, 2P < 0.001, vs. AS group; aP < 0.05, bP < 0.001, vs. Simva group;\*P < 0.05, \*\*P < 0.001, vs. L-4F group. Image collected & cropped by CiteAb from the following publication (https://lipidworld.biomedcentral.com/articles/10.1186/1476-511X-12-180), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



SR-B

SEC



W-T=AS=Sim





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Actin

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Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free C [NB400-104] - Identification of Plasmodium sporozoite-associated receptors on immortalized hepatocyte-like cells (imHCs). The expression of Plasmodium sporozoite-associated receptors (CD81, EphA2 & SR-BI) on imHCs & HC-04 cells was determined using immunofluorescent staining. Prior to analysis, the hepatocytes were grown in MEM/F12 medium containing 10% fetal bovine serum until confluence was reached. The expression of CD81 (a), EphA2 (b) & SR-BI (c) on imHCs & HC-04 cells was compared (d, e, f, respectively). Hepatocyte nuclei were stained with Hoechst 33342 dye. Fluorescence images were captured & analysed using an Operetta High-Content Imaging System (PerkinElmer) with a ×40 objective lens. Scale bar = 50 µm. Cells expressing CD81, EphA2 or SR-BI were quantified from 15 randomly selected image fields (total number of analysed cells > 2000) (g, h, i, respectively). Bar graph shows the mean percentage of positively stained cells. Error bars depict standard deviations. \*\*\*\*p < 0.0001, Student's t test Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29370800), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free [NB400-104] - Immunofluorescence assay on GIM biopsies of HCVtransplanted patients. Immunofluorescence assay on GIM polyp biopsies of HCV-transplanted patients, using antibodies against HCV receptors: CD81, SR-B1, Claudin-1, Occludin (Original magnification X400). (A, E) CD81 in colon (patient 26); (B, F) Claudin-1 in colon (patient 26); (C, G) Occludin in colon (C patient 28, G patient 26); (D) SR-B1 in antrum (patient 28); (H) SR-B1 in colon (patient 26). All the sections were clearly positive for the analyzed HCV receptors before (A-D) & after transplantation (E-H). Negative controls (I-N) were performed on GIM biopsies of HCV positive patients by omitting the primary antibodies, & by using polyclonal FITC-conjugated Donkey anti-mouse (I) & anti-rabbit (L, M, N) as secondary antibodies. (I CD81 in antrum; L Claudin-1 in polyp colon; M Occludin in antrum; N SR-B1 in polyp colon. Nuclei were counterstained with DAPI (blue). Scale bar 50 µm. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0181683), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Immunocytochemistry/ Immunofluorescence: SR-BI Antibody - BSA Free C [NB400-104] - ALDH2 rs671 modulates HDL-C levels in mice & human liver through increasing poly(ADP-ribosyl)ation of LXRa due to attenuated ALDH2/PARP1 interaction.(A) Representative H&E & Oil Red O staining for mouse liver fed with a Western diet (WD) for 8 weeks (WT & ALDH2 rs671-KI mice, referred to as rs671). Scale bar: 100 µm. (B) HDL-C in plasma at 16th week (WD for 8 weeks). WT, n = 9; rs671, n = 10. (C) Western blotting analysis of ABCA1, ALDH2, LXRa, & SR-B1 expression in mouse liver tissue. WT, n = 3; rs671, n = 3. (D) IP results of WT ALDH2 or ALDH2 rs671, PARP1. (E) IP results of ALDH2 & PARP1 in human liver tissues. WT, n = 3; rs671, n = 3. Experiments were repeated 3 times. (F) ALDH2 rs671 significantly increased nuclear translocation of PARP1 in human liver tissue. WT, n = 3; rs671, n = 3. Statistical comparisons were made using a 2-tailed Student's t test. All data are mean ± SD. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35393951), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





 Pre-OLT biopsies
 Post-OLT biopsies
 Negative control

 CDe1
 Image: CDe1 mark
 Image: CDe1 mark





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Western Blot: SR-BI Antibody - BSA Free [NB400-104] - Differentiation & characteristics of iHLCs. (a) Cell morphology & culture conditions at different stages of differentiation from iPSCs into iHLCs. Bright-field images were taken at the indicated time points (scale bar  $100 \,\mu$ m). (b) Sequential expression & repression of transcription factors marking successful differentiation. Cells were immunostained with antibodies against Oct 3/4 (pluripotency marker), the endodermal transcription factor GATA-4, & the hepatic marker HNF4a at day 0, 5, & 10 of differentiation (scale bars 20 µm). Shown are representative fluorescence & DIC images. (c) mRNA expression levels of different hepatocyte-specific factors in iHLCs, Huh7.5 cells, & primary human hepatocytes (PHHs) were analysed via qRT-PCR. Expression levels are displayed as fold over iPSCs normalised to GAPDH & 18S rRNA (mean  $\pm$  SEM, n = 3–4, \*p < 0.05). (d) Western blot analysis of protein expression in HCV-infected iHLCs compared to Huh7.5 cells of different host factors crucial for HCV infection. Tubulin served as loading control. Full-length blots are presented in Supplementary Figure 1. (e) iHLCs & iPSCs were immunostained for the hepatic marker HNF4α & the tight junction proteins occludin & claudin-1 (nuclei were visualised with Drag5) (scale bar 20 µm). (f) Metabolic functionality of mature iHLCs was evaluated by analysing glycogen storage by Periodic acid-Schiff (PAS) staining & indocyanine green (ICG) uptake visualised by bright-field microscopy (scale bar 100 µm). Shown are representative bright-field images. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29497123), licensed under a CC-BY license. Not internally tested by Novus Biologicals. Western Blot: SR-BI Antibody - BSA Free [NB400-104] - Evodiamine enhances apo A1-mediated ChE from THP-1 macrophages & increases ABCA1 protein level. (a) Differentiated THP-1 cells were loaded with [3H]-cholesterol together with the indicated treatments for 24 h. On the next day, the cells were washed twice with PBS & incubated with the same compounds [solvent vehicle control (Veh; ≤0.1% DMSO), evodiamine  $(1-20 \mu M)$ , & the PPARy agonist pioglitazone  $(10 \mu M)$  as positive control] with or without 10 µg/mL apo A1. Extracellular as well as intracellular radioactivities were quantified with scintillation counter. Differentiated THP-1-derived macrophages were treated with solvent vehicle control (Veh;  $\leq 0.1\%$  DMSO), evodiamine (10  $\mu$ M), & the PPARy agonist pioglitazone (10 µM) as positive control. After 24 h incubation,

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the cells were lysed & 20 µg protein was resolved via SDS-PAGE. Immunodetection was performed with antibodies against the indicated

chemiluminescence detection. All experiments were performed at least

three times & data are presented as means ± S.D. vs. solvent vehicle

(ANOVA/Bonferroni). Image collected & cropped by CiteAb from the

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proteins, ABCA1 (b), ABCG1 (c), & SR-B1 (d), & visualized by

control, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, n.s. no significance



Western Blot: SR-BI Antibody - BSA Free [NB400-104] - siRNA-Е mediated knock down of SR-B1 impairs HDL-stimulated AKT phosphorylation in HIVCMs.(A) Representative immunoblots of phospho-AKT (pAKT), total AKT (tAKT), & β-actin (loading control) & (B) quantification of the ratio of pAKT/tAKT (n = 3) in HIVCMs incubated with HDL (100 µg protein/ml) for different times. (C) Representative immunoblots of pAKT & tAKT & (D) quantification of the ratio of pAKT/tAKT (n = 3) in HIVCMs incubated with HDL (100 µg protein/ml) for 30 min prior to & during exposure to OGD or maintenance under normal conditions ('Normoxia'). (E) Representative immunoblots of pAKT, tAKT, SR-B1, & β-actin (loading control) in siCntrl & siSR-B1-transfected HIVCMs incubated with or without HDL for 30 min. (F) Quantification of the ratio of pAKT/tAKT (expressed as fold change relative to untreated; n = 4). Data are means ± SEM. \*\*P < 0.004; \*P < 0.05; ns, not statistically significant (P > 0.9) by one-way ANOVA. §P < 0.02 by unpaired Student's t-test, relative to 0 h time point. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29523748), licensed under a CC-BY

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#### **Publications**

Cabodevilla AG, Tang S, Lee S, Mullick AE et Al. Eruptive xanthoma model reveals endothelial cells internalize and metabolize chylomicrons, leading to extravascular triglyceride accumulation J Clin Invest 2021-06-15 [PMID: 34128469]

Su JH, Hong Y, Han CC, Yu J et Al. Dual action of macrophage miR-204 confines cyclosporine A-induced atherosclerosis Br J Pharmacol 2023-09-13 [PMID: 37702564]

Zhu L, Litts B, Wang Y et AI. Ablation of IFN? in myeloid cells suppresses liver inflammation and fibrogenesis in mice with hepatic small heterodimer partner (SHP) deletion Mol Metab 2024-04-06 [PMID: 38589002] (Western Blot)

Ivone Cristina Igreja Sá, Katarina Tripska, Milos Hroch, Radomir Hyspler, Alena Ticha, Hana Lastuvkova, Jolana Schreiberova, Eva Dolezelova, Samira Eissazadeh, Barbora Vitverova, Iveta Najmanova, Martina Vasinova, Miguel Pericacho, Stanislav Micuda, Petr Nachtigal Soluble Endoglin as a Potential Biomarker of Nonalcoholic Steatohepatitis (NASH) Development, Participating in Aggravation of NASH-Related Changes in Mouse Liver International Journal of Molecular Sciences 2020-11-27 [PMID: 33261044]

Li J, Cai X, Yang Y et Al. Macrophage MST1 protects against schistosomiasis-induced liver fibrosis by promoting the PPAR?-CD36 pathway and suppressing NF-?B signaling PLoS Pathog 2024-12-19 [PMID: 39700261]

Rodriguez PJ, Gillard BK, Barosh R et Al. Neo High-Density Lipoprotein Produced by the Streptococcal Serum Opacity Factor Activity against Human High-Density Lipoproteins Is Hepatically Removed via Dual Mechanisms Biochemistry 2017-05-09 [PMID: 27662183] (Western Blot)

Han W, Zhang D, Zhang P et Al. Danlou Recipe promotes cholesterol efflux in macrophages RAW264.7 and reverses cholesterol transport in mice with hyperlipidemia induced by P407 BMC Complement Med Ther 2023-12-08 [PMID: 38066464]

Zhu L, An J, Chinnarasu S et al. Expressing the Human Cholesteryl Ester Transfer Protein Minigene Improves Diet-Induced Fatty Liver and Insulin Resistance in Female Mice Frontiers in physiology 2022-01-10 [PMID: 35082691] (Western Blot)

Chi H, Qu B, Prawira A et al. An hepatitis B and D virus infection model using human pluripotent stem cell-derived hepatocytes EMBO Reports 2024-09-04 [PMID: 39232200]

Christine Bassila, George E.G. Kluck, Narmadaa Thyagarajan, Kevin M. Chathely, Leticia Gonzalez, Bernardo L. Trigatti Ligand-dependent interactions between SR-B1 and S1PR1 in macrophages and atherosclerotic plaques Journal of Lipid Research 2024-04-05 [PMID: 38583587]

Qijun Chen, Lixue Wang, Hui Song, Wen Xing, Junfeng Shi, Yudi Li, Ziqian Wang, Jinlong Chen, Nan Xie, Wenhua Zhao Deficiency of SR B1 reduced the tumor load of colitis induced or APC min /+ induced colorectal cancer Cancer Medicine 2023-09-28 [PMID: 37766594]

Alejandra Vargas-Caraveo, Aline Sayd, Sandra R. Maus, Javier R. Caso, José L. M. Madrigal, Borja García-Bueno, Juan C. Leza Lipopolysaccharide enters the rat brain by a lipoprotein-mediated transport mechanism in physiological conditions Scientific Reports 2017-10-13 [PMID: 29030613]

More publications at http://www.novusbio.com/NB400-104



#### Procedures

Western Blot protocol for SR-BI Antibody (NB400-104) 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.

# Immunocytochemistry/ Immunofluorescence Protocol for SR-BI Antibody (NB400-104)

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.



#### Immunohistochemistry-Paraffin Protocol for SR-BI Antibody (NB400-104)

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.



#### Flow (Intracellular) Protocol for SR-BI Antibody (NB400-104)

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 NB400-104

NBL1-15720	SR-BI Overexpression Lysate
NB400-104PEP-0.1mg	SR-BI 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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