Product Datasheet

ABCG1 Antibody - BSA Free

NB400-132

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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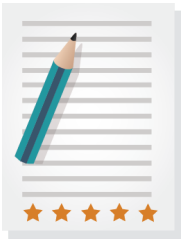
Updated 10/23/2024 v.20.1



NB400-132

ABCG1 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	75.59 kDa

Product Description

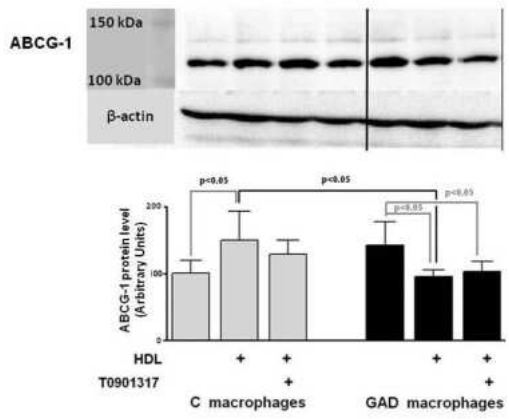
Host	Rabbit
Gene ID	9619
Gene Symbol	ABCG1
Species	Human, Mouse, Rat, Chinese Hamster, Hamster, Monkey, Rabbit
Reactivity Notes	Rabbit reactivity reported in scientific literature (PMID: 23185679). Chinese Hamster and Monkey reactivity reported in scientific literature (PMID: 27230131).
Immunogen	A synthetic peptide made to an internal region of human ABCG1 (between residues 300-400). [UniProt# P45844]

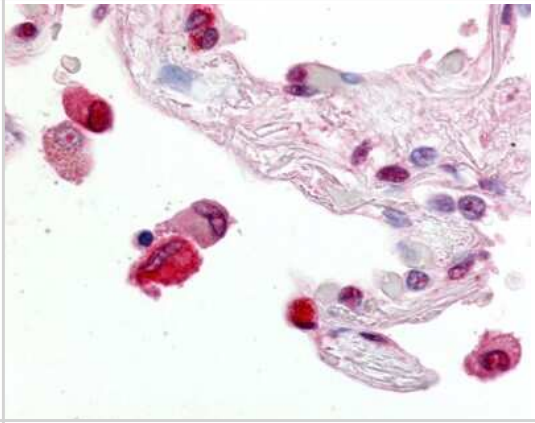
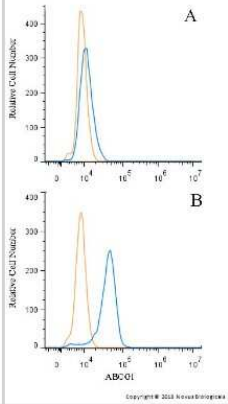
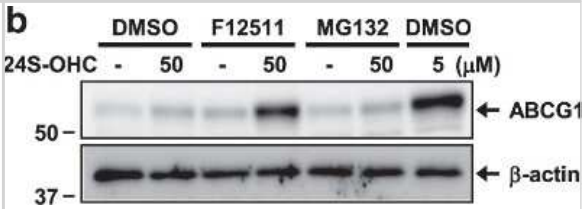
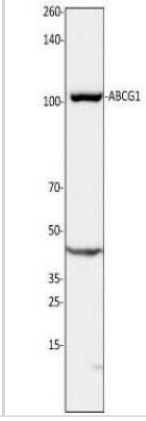
Product Application Details

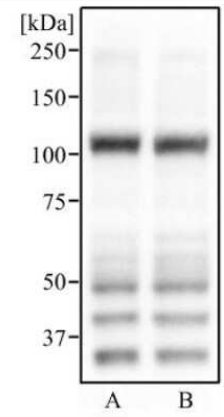
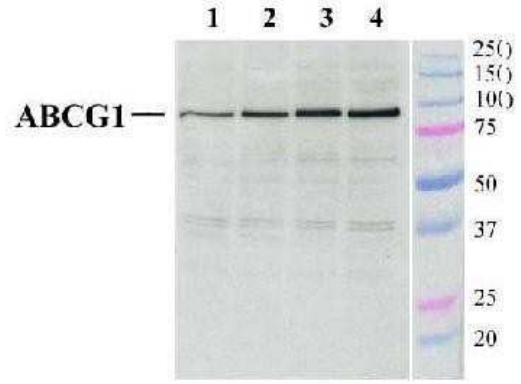
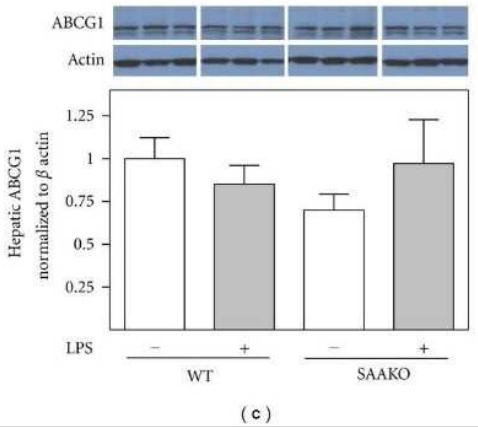
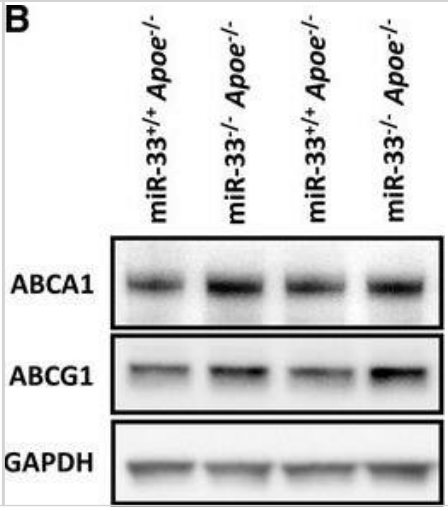
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, In vitro assay, In vivo assay, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500, Flow Cytometry, Immunohistochemistry 1:100-1:400, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 23185679), Immunoprecipitation reported in scientific literature (PMID 30235209), Immunohistochemistry-Paraffin 1:100-1:400, Immunoblotting reported in scientific literature (PMID 28264879), In vitro assay, In vivo assay reported in scientific literature, Flow (Intracellular)

Images

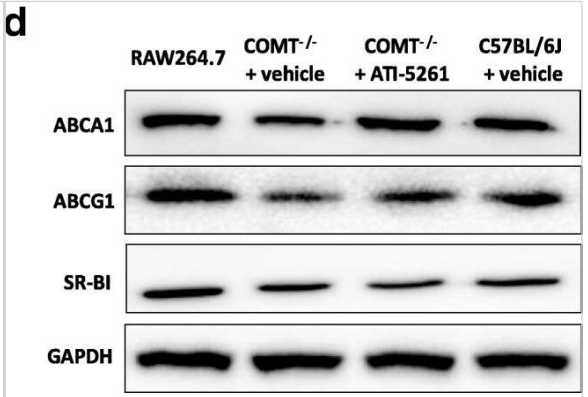
Western Blot: ABCG1 Antibody - BSA Free [NB400-132] - ABCG-1 protein level in control and glycolaldehyde-treated macrophages. J774 macrophages were overloaded with oxidized LDL (50 ug/mL de DMEM) for 48 h. In the last 5h, cells were incubated with 0.25 mM glycolaldehyde (GAD; black bars) and 1 uM LXR agonist (T0901317). Control cells (C; gray bars) were incubated with T0901317 alone. Cells were scrapped into TBS containing protease inhibitors. Equal amounts of cell protein were applied into a 6% polyacrylamide electrophoresis gel and immunoblot was performed utilizing anti ABCG-1 antibody. Data (arbitrary units; n = 4) were corrected per beta-actin. Image collected and cropped by CiteAb from the following publication (https://lipidworld.biomedcentral.com/articles/10.1186/1476-511X-10-172), licensed under a CC-BY license.



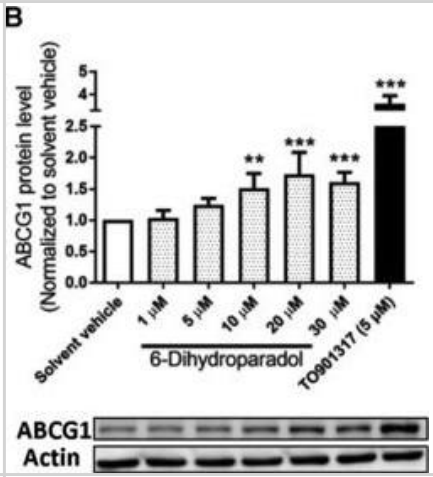
Immunohistochemistry-Paraffin: ABCG1 Antibody - BSA Free [NB400-132] - Staining of human lung, alveolar macrophages.	
Flow (Intracellular): ABCG1 Antibody - BSA Free [NB400-132] - RAW 246.7 cells were either untreated (A) or serum starved for 24 hours, and then treated with 1uM TO9 for 24 hours (B). An intracellular stain was performed with NB400-132 (blue) and a matched isotype control NB810-56910 (orange). Cells were fixed with 4% paraformaldehyde, following fixation, cells were permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature, followed by Goat Anti-Rabbit Dylight 550-conjugated antibody.	
Western Blot: ABCG1 Antibody - BSA Free [NB400-132] - Cells were pretreated with 5 uM F12511 or 5 uM MG132 for 30 min and then exposed to 5 or 50 uM 24S-OHC for 6 h. Whole-cell lysates were immunoblotted with antibodies specific for ABCG1 or beta-actin. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/31285856/) licensed under a CC-BY license.	
Western Blot: ABCG1 Antibody - BSA Free [NB400-132] - Detection of ABCG1 in J774 mouse macrophages. Image provided by Dr. Angeliki Chroni of the National Centre for Scientific Research Demokritos.	

Western Blot: ABCG1 Antibody - BSA Free [NB400-132] - Raw 264.7 (A), and Raw 264.7 + 10uM T09 18 hr (B) cell lysate using ABCG1 antibody at 2 ug/mL.	
Western Blot: ABCG1 Antibody - BSA Free [NB400-132] - Detection of ABCG1 in mouse peritoneal macrophages. Lane 1: untreated mouse peritoneal macrophages, Lane 2: 22-(R)-hydrocholesterol treated macrophages, Lane 3: T0901713 treated macrophages, Lane 4: T091713 treated macrophages.	
Western Blot: ABCG1 Antibody - BSA Free [NB400-132] - SAA has little impact on the impairment in biliary and fecal excretion of macrophage-derived 3H-cholesterol during endotoxemia. WT and SAAKO mice were injected subcutaneously with PBS or 0.8 ug/g LPS as indicated, followed 4 hr later by intraperitoneal injection of 3H-cholesterol-labeled J774 macrophages. ABCG1 in liver lysates (40 ug protein) was identified by immunoblotting and quantified by densitometry. Results from each of the 3 experiments (n = 4-5 per group per experiment) were expressed relative to PBS-injected WT mice after normalization to beta-actin. A representative western blot is shown. Image collected and cropped by CiteAb from the following publication (https://www.hindawi.com/journals/jl/2013/283486/), licensed under a CC-BY license.	
miR-33 deficiency improved cholesterol efflux in macrophages. A, Quantitative real-time PCR analysis of Abca1 & Abcg1 in macrophages from miR-33+/+Apoe−/− & miR-33−/−Apoe−/− mice. Values from miR-33+/+Apoe−/− were set at 100%. Values are mean±SE (n=7 each); ***P<0.001. B, Western blotting analysis of ABCA1 & ABCG1 in thioglycollate-elicited peritoneal macrophages from miR-33+/+Apoe−/− & miR-33−/−Apoe−/− mice. GAPDH was used as a loading control. C, Cholesterol efflux from thioglycollate-elicited peritoneal macrophages in the presence or absence of apoA-I (5 or 10 μg/mL). Values are mean ±SE (n=6 each); **P<0.01. D, Cholesterol efflux from thioglycollate-elicited peritoneal macrophages in the presence or absence of HDL-C (50 or 100 μg/mL). Values are mean±SE (n=6 each); **P<0.01. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23316322), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

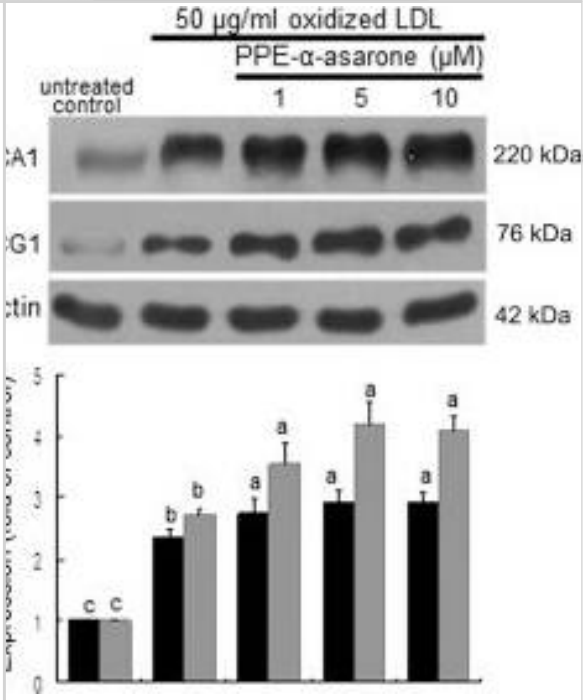
ABCA1, ABCG1 & SR-BI protein expressions. a & b Hepatic protein expressions of ABCA1, ABCG1 & SR-BI were significantly decreased in COMT−/− mice at GD 18.5, compared to C57BL/6 J mice. Decreased hepatic ABCA1 expression was also observed at 10 days postpartum. ATI-5261 increased ABCA1 & ABCG1 expression in the liver at 10 days postpartum. c Placental protein expressions of ABCA1 & ABCG1 was reduced in COMT−/− mice, compared to C57BL/6 J mice. ATI-5261 treatment significantly increased ABCA1 levels in the placenta of COMT−/− mice. d Representative immunoblots of the corresponding proteins in the placenta with mouse RAW264.7 cell lysate included as positive control. Similar results were obtained when the experiment was repeated with lysates prepared from three batches of tissues. Data are presented as mean ± SEM. Groups (n = 8 in all groups) were compared using one-way ANOVA with post-hoc analysis (Tukey's procedure). *, p < 0.05 Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30237900), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



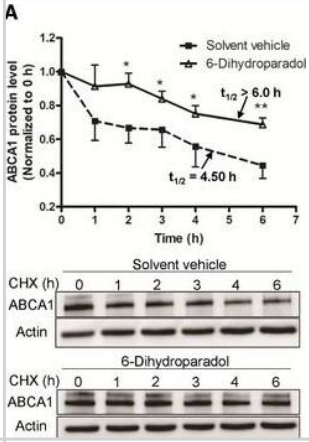
6□Dihydroparadol increases A) ABCA1 & B) ABCG1, but not C) SR□BI protein levels in cholesterol□loaded THP□1□derived macrophages. THP□1 cells were differentiated as described in Figure 2, & then loaded with unlabeled cholesterol for 24 h. Cells were treated with increasing concentrations of 6□dihydroparadol (1–30 μm) for another 24 h. The protein levels of ABCA1, ABCG1, & SR□B1 were detected by Western blot analysis. The control was treated with solvent vehicle (0.1% DMSO). As a positive control, TO901317 (5 μm, 24 h) was used. The bar graphs represent mean ± SD from three independent experiments. *p < 0.05, **p < 0.01, & ***p < 0.001 versus control (determined by Student's t□test or ANOVA with Bonferroni's post hoc test). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29802792), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



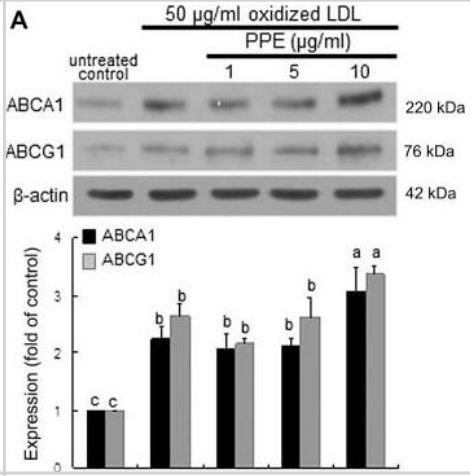
(A) Chemical structure, (B) cytotoxicity of α-asarone, (C) enhancement of cholesterol efflux by α-asarone, (D & E) upregulation of ABCA1 & ABCG1 by α-asarone & β-asarone, & (F) elevation of retinoid X receptor (RXR)α transcription. J774A.1 murine macrophages were exposed to 50 μg/ml oxidized low-density lipoprotein (LDL) & treated with 1–10 μM purple Perilla frutescens extracts (PPE)-α-asarone & 5–10 μM β-asarone. (B) MTT assay was performed for the measurement of α-asarone toxicity. Graph data represent 1 of 4 independent experiments with multiple estimations. Values are expressed as the percentage cell survival relative to the untreated control cells (cell viability, 100%). (C) Cholesterol efflux was expressed as the percentage fluorescence in the medium relative to total fluorescence. (D & E) For the measurement of ABCA1 & ABCG1 expression, total cell lysates were subjected to western blot analysis with a primary antibody against ABCG1 or ABCG1. β-actin was used as an internal control. Bar graphs (means ± SEM, n=3) represent quantitative densitometric results of the upper bands. Bar graphs denoted without a common letter indicate significant difference, P<0.05. (F) RXRα mRNA expression was measured by RT-PCR. GAPDH was used as a housekeeping gene for the co-amplification with RXRα. Image collected & cropped by CiteAb from the following publication (https://www.spandidos-publications.com/10.3892/ijmm.2015.2101), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



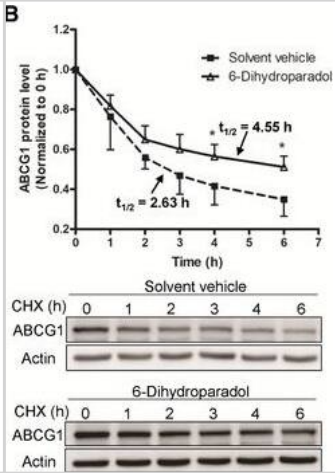
6□Dihydroparadol increases A) ABCA1 & B) ABCG1 protein stability. THP□1 cells were differentiated as described in Figure 2. Then cells were loaded with unlabeled cholesterol & treated with 6□dihydroparadol (10 µm), or solvent vehicle (0.1% DMSO, control) for 24 h. Cells were lysed at different time points (0, 1, 2, 3, 4, & 6 h) after treatment with the protein synthesis inhibitor cycloheximide (CHX, 100 µm). The protein levels of both ABCA1 & ABCG1 were detected by Western blot analysis. The data points represent mean ± SD from three independent experiments. *p < 0.05 & **p < 0.01 versus control at the same time point (determined by Student's t□test). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29802792), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



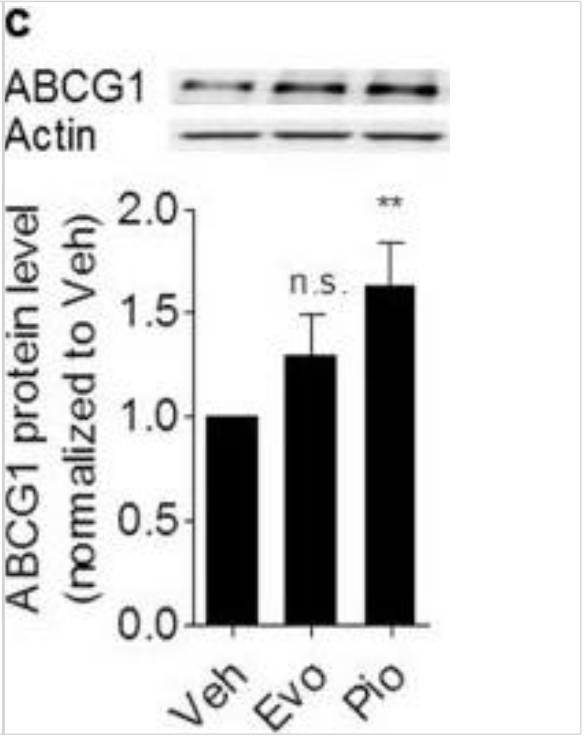
(A) Upregulation of ABCA1 & ABCG1 & (B) enhancement of cholesterol efflux by purple Perilla frutescens extracts (PPE) in 50 µg/ml Cu2+-oxidized low-density lipoproteins (LDL)-exposed J774A.1 murine macrophages. (A) For the measurement of ABCA1 & ABCG1 expression, total cell lysates were subjected to western blot analysis with a primary antibody against ABCA1 or ABCG1. β-actin was used as an internal control. Bar graphs (means ± SEM, n=3) represent quantitative densitometric results of the upper bands. (B) Cholesterol efflux was expressed as the percentage of fluorescence in the medium relative to the total fluorescence. Bar graphs denoted without a common letter indicate significant difference, P<0.05. Image collected & cropped by CiteAb from the following publication (https://www.spandidos-publications.com/10.3892/ijmm.2015.2101), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: ABCG1 Antibody - BSA Free [NB400-132] - 6□Dihydroparadol increases A) ABCA1 & B) ABCG1 protein stability. THP□1 cells were differentiated as described in Figure 2. Then cells were loaded with unlabeled cholesterol & treated with 6□dihydroparadol (10 µm), or solvent vehicle (0.1% DMSO, control) for 24 h. Cells were lysed at different time points (0, 1, 2, 3, 4, & 6 h) after treatment with the protein synthesis inhibitor cycloheximide (CHX, 100 µm). The protein levels of both ABCA1 & ABCG1 were detected by Western blot analysis. The data points represent mean ± SD from three independent experiments. *p < 0.05 & **p < 0.01 versus control at the same time point (determined by Student's t□test). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29802792), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: ABCG1 Antibody - BSA Free [NB400-132] - 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 μM), & the PPARγ agonist pioglitazone (10 μM) 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; ≤0.1% DMSO), evodiamine (10 μM), & the PPARγ agonist pioglitazone (10 μM) as positive control. After 24 h incubation, the cells were lysed & 20 μg protein was resolved via SDS-PAGE. Immunodetection was performed with antibodies against the indicated proteins, ABCA1 (b), ABCG1 (c), & SR-B1 (d), & visualized by chemiluminescence detection. All experiments were performed at least three times & data are presented as means ± S.D. vs. solvent vehicle control, *p < 0.05, **p < 0.01, ***p < 0.001, n.s. no significance (ANOVA/Bonferroni). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30038271), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Zhang Y, Li H, Huang Y et al. Stage-Dependent Impact of RIPK1 Inhibition on Atherogenesis: Dual Effects on Inflammation and Foam Cell Dynamics Frontiers in Cardiovascular Medicine 2021-10-25 [PMID: 34760938] (Flow Cytometry)

Giorgia Centonze, Dora Natalini, Silvia Grasso, Alessandro Morellato, Vincenzo Salemme, Alessio Piccolantonio, Giacomo D'Attanasio, Aurora Savino, Olga Teresa Bianciotto, Matteo Fragomeni, Andrea Scavuzzo, Matteo Poncina, Francesca Nigrelli, Mario De Gregorio, Valeria Poli, Pietro Arina, Daniela Taverna, Joanna Kopecka, Sirio Dupont, Emilia Turco, Chiara Riganti, Paola Defilippi p140Cap modulates the mevalonate pathway decreasing cell migration and enhancing drug sensitivity in breast cancer cells Cell Death & Disease 2023-12-20 [PMID: 38123597]

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]

Huang R, Hu Z, Chen X Et al. The Transcription Factor SUB1 Is a Master Regulator of the Macrophage TLR Response in Atherosclerosis Advanced science (Weinheim, Baden-Wurttemberg, Germany) 2021-08-10 [PMID: 34378353]

Liu Q, Xiao JJ, Wang S et al. Paraoxonase 1 Ameliorates Renal Lipotoxicity by Activating Lipophagy and Inhibiting Pyroptosis The American journal of pathology 2022-08-10 [PMID: 35963464]

Oladosu O, Esobi IC, Powell RR et al. Dissecting the Impact of Vascular Smooth Muscle Cell ABCA1 versus ABCG1 Expression on Cholesterol Efflux and Macrophage-like Cell Transdifferentiation: The Role of SR-BI Journal of cardiovascular development and disease 2023-10-02 [PMID: 37887863] (WB)

Ram□rez CM, Torrecilla-Parra M, Pardo-Marqu□s V et al. Crosstalk Between LXR and Caveolin-1 Signaling Supports Cholesterol Efflux and Anti-Inflammatory□Pathways□in Macrophages Front Endocrinol (Lausanne) 2021-05-27 [PMID: 34122329]

Xu H, Zheng LX, Chen XS et al. Brain-specific loss of Abcg1 disturbs cholesterol metabolism and aggravates pyroptosis and neurological deficits after traumatic brain injury Brain Pathology 2023-05-01 [PMID: 36271611] (Immunohistochemistry, Immunocytochemistry/ Immunofluorescence)

Esobi I, Olanrewaju O, Echesabal-Chen J, Stamatikos A. Utilizing the LoxP-Stop-LoxP System to Control Transgenic ABC-Transporter Expression In Vitro Biomolecules 2022-05-08 [PMID: 35625607]

Miroshnikova VV, Panteleeva AA, Bazhenova EA et al. [Regulation of ABCA1 and ABCG1 gene expression in the intraabdominal adipose tissue] Biomeditsinskaya Khimiya 2016-07-16 [PMID: 27420620]

Liu S, Zhang Y, Zheng X et al. Sulforaphane Inhibits Foam Cell Formation and Atherosclerosis via Mechanisms Involving the Modulation of Macrophage Cholesterol Transport and the Related Phenotype Nutrients 2023-04-28 [PMID: 37432260] (Immunohistochemistry-Frozen, Immunocytochemistry/ Immunofluorescence)

Prasad R, Adu-Agyeiwaah Y, Floyd JL et al. Sustained ACE2 Expression by Probiotic Improves Integrity of Intestinal Lymphatics and Retinopathy in Type 1 Diabetic Model Journal of clinical medicine 2023-02-23 [PMID: 36902558] (IHC-P, Mouse)

More publications at http://www.novusbio.com/NB400-132

Procedures

Western Blot protocol for ABCG1 Antibody (NB400-132)

Western Blot Protocol for NB 400-132
Protein Extraction:
1. After washing with PBS, cells (mouse peritoneal macrophages grown in a 60 mm dish) in 300 ml of cold lysis buffer [50 mM Tris, pH 7.5, 100 mM NaCl, 1% Triton x-100, 1% NaC24H39O4, 50 mM NaF, 1 mM Na3VO4, 1 mM PMSF, 50 ug/ml of aprotinin and 50 ug/ml of leupeptin] are lysed by scraping and sonicating for 25 seconds on ice.
2. Spin cellular lysate for 10 min. at 13,000 rpm at 4 degrees Celcius.
3. Save supernatant and store at -20 degrees Celcius.
Western blotting:
1. Determine protein content by Lowry method.
2. Load 40 ug of cellular protein [pre-boiled for 5 min. in sample buffer] on a 7.5% SDS-PAGE separating gel.
3. Run electrophoresis for 90 min. at RT in 1x electrophoresis buffer.
4. After electrophoresis, equilibrate the gel and nitrocellulose membrane in transfer buffer.
5. Transfer proteins in 1x transfer buffer for 1 hour at 100 volts and RT.
6. Block the membrane in 10 ml of TBS with 5% NFDM for 1 hour at RT.
7. After a quick rinse with TBS-T (0.5% Tween-20), membrane is incubated in diluted anti-ABCG1 (cat# NB 400-132) in 1% NFDM/TBS for 1.5 hours at RT.
8. Wash the membrane in 25 ml of TBS-T for 3x 5 minutes at RT.
9. Incubate the membrane in 10 ml of diluted secondary antibody (Anti-Rabbit IgG-HRP Conjugate) in 1% NFDM/TBS for 1 hour at RT.
10. Wash the membrane with 25 ml of TBS-T for 3x 5 minutes at RT.
11. Incubate the membrane in ECL Western blotting detection reagents for 1 minute.
12. Expose to film for ~2 minutes (adjust time as needed for best image).

Immunohistochemistry-Paraffin protocol for ABCG1 Antibody (NB400-132)

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:
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 (do not wash off the Blocking Reagent), 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.
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 1/2 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).

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