

Product Datasheet

ABCA1 Antibody NB400-105SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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NB400-105SS

ABCA1 Antibody

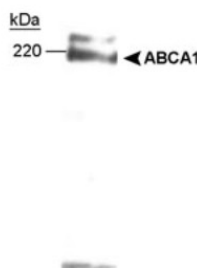
Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-citrate/phosphate, pH 7-8
Target Molecular Weight	220 kDa

Product Description	
Host	Rabbit
Gene ID	19
Gene Symbol	ABCA1
Species	Human, Mouse, Rat, Canine, Chicken, Hamster, Mustelid, Porcine
Species Reactivity	Human, hamster, mouse, rat, porcine, chicken and mustelid. Canine reactivity reported in scientific literature (PMID: 24612239)
Immunogen	Partial peptide sequence (within residues 1100-1300) of human ABCA1 [UniProt# O95477]. Actual immunogen sequence is proprietary information.

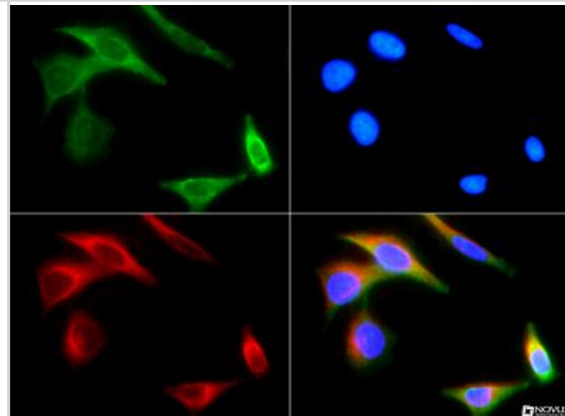
Product Application Details	
Applications	Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Chromatin Immunoprecipitation, ELISA, Flow Cytometry 1:400, Immunocytochemistry/Immunofluorescence 1:100, Immunohistochemistry 1:200, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin 1:200, Immunoprecipitation 1:10-1:500, Simple Western 1:50, Western Blot 1:500
Application Notes	This ABCA1 antibody is useful for Flow Cytometry, Immunocytochemistry/Immunofluorescence (PMID: 21501868), Immunoprecipitation (PMID: 21846716; 21106522), Western blot and Immunohistochemistry on frozen and paraffin embedded sections. In Western blot a band representing ABCA1 is observed at ~220 kDa. Additional non-specific bands are seen at lower molecular weights, but do not interfere with the ABCA1 signal. NOTE: It is important not to boil the sample before loading onto a gel. Boiling can cause aggregation in large proteins, resulting in the proteins inability to enter the gel. Chromatin Immunoprecipitation and ELISA were reported in scientific literature. In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

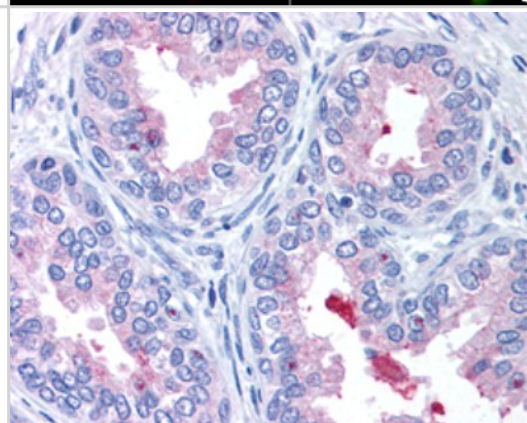
Western Blot: ABCA1 Antibody [NB400-105] - Analysis of ABCA1, using NB400-105. Samples: 40 ug of total cell post-nuclear lysate of raw macrophages treated with 9-cisretinoic acid and 22R-hydroxycholesterol.



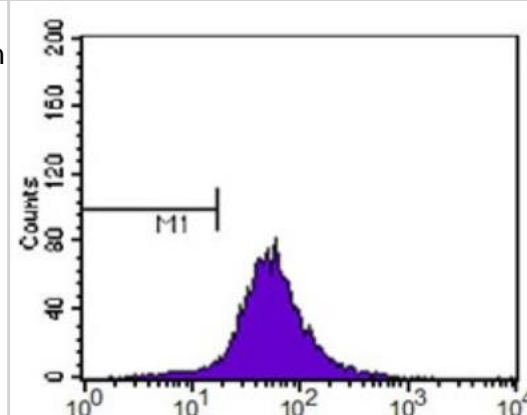
Immunocytochemistry/Immunofluorescence: ABCA1 Antibody [NB400-105] - ABCA1 antibody was tested at 1:100 in HeLa cells with FITC (green). alpha-Tubulin antibody NB100-690 was tested at a 1:500 dilution using Mouse IgG secondary Antibody NB710-94914 at 1:1000 [HiLyte Fluor 555] (red). Nuclei were counterstained with Dapi (blue).



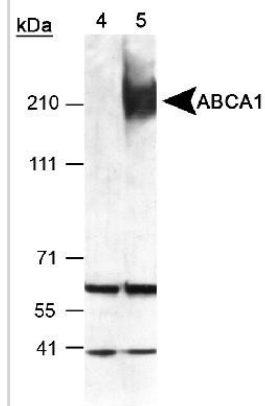
Immunohistochemistry: ABCA1 Antibody [NB400-105] - Detection of ABCA1 in prostate epithelium showing luminal and membrane staining.



Flow Cytometry: ABCA1 Antibody [NB400-105] - ABCA1 antibody was tested at 1:400 in HeLa cells using an Alexa Fluor 488 secondary (shown in purple). M1 is defined by unstained cells.



Western Blot: ABCA1 Antibody [NB400-105] - Detection of ABCA1 in mouse peritoneal macrophages using NB 400-105 (Lot L). ECL exposure, 1 min. Lane 4: T09 uninduced lysate Lane 5: T09 induced lysate.



Publications

De Boussac H, Maqdasy S, Trousson A et al. Enolase is regulated by Liver X Receptors Steroids. 2015 Feb 21 [PMID: 25708389] (WB, Mouse)

Park SH, Paek JH, Shin D et al. Purple perilla extracts with alpha-asarone enhance cholesterol efflux from oxidized LDL-exposed macrophages Int. J. Mol. Med. 2015 Feb 12 [PMID: 25673178] (WB, Mouse)

Hong C, Marshall Sm, Mcdaniel Al et al. The LXR-Idol Axis Differentially Regulates Plasma LDL Levels in Primates and Mice. Cell Metab. 2014 Nov 04 [PMID: 25440061]

Shibuya Y, Chang Cc, Huang Lh et al. Inhibiting ACAT1/SOAT1 in Microglia Stimulates Autophagy-Mediated Lysosomal Proteolysis and Increases AB1-42 Clearance. J. neurosci. 2014 Oct 22 [PMID: 25339759] (WB, Mouse)

Dong F, Mo Z, Eid W et al. Akt Inhibition Promotes ABCA1-Mediated Cholesterol Efflux to ApoA-I through Suppressing mTORC1. PLoS One. 2014 Nov 22 [PMID: 25415591] (WB, Hamster)

Kuo CH, Leon L, Chung EJ, Huang RT. Inhibition of atherosclerosis-promoting microRNAs via targeted polyelectrolyte complex micelles. Journal of Materials Chemistry B. 2014 Sep 05

Tai LM, Koster KP, Luo J et al. Amyloid-b Pathology and APOE Genotype Modulate Retinoid X Receptor Agonist Activity in vivo. J Biol Chem. 2014 Sep 12 [PMID: 25217640]

Xu Y, Liu Q, Xu Y et al. Rutaecarpine suppresses atherosclerosis in ApoE^{-/-} mice through upregulating ABCA1 and SR-BI within RCT. J Lipid Res. 2014 Jun 7 [PMID: 24908654]

Liu W, Jiang J, Yan D et al. Pentraxin 3 promotes oxLDL uptake and inhibits cholesterol efflux from macrophage-derived foam cells. Exp. Mol. Pathol. 3/24/2014 [PMID: 24675235] (WB, Human)

Sallam T, Ito A, Rong X et al. The macrophage lipopolysaccharide binding protein gene is an LXR target that promotes macrophage survival and atherosclerosis. J. Lipid Res. 3/26/2014 [PMID: 24671012] (WB, Mouse)

Bocchetta S, Maillard P, Yamamoto M et al. Up-Regulation of the ATP-Binding Cassette Transporter A1 Inhibits Hepatitis C Virus Infection. PLoS ONE 3/20/2014 [PMID: 24646941] (WB, Human)

Du RH, Qin SY, Shi LS et al. Fumigaclavine C activates PPARgamma pathway and attenuates atherogenesis in ApoE-deficient mice. Atherosclerosis 3/5/2014 [PMID: 24637412] (WB, Mouse)

More publications at <http://www.novusbio.com/NB400-105>

Procedures

Western Blot Protocol for ABCA1 Antibody (NB400-105)

Western Blot

The experiment was performed by treating RAW macrophages with 9-cis-retinoic acid and 22R-hydroxycholesterol, known inducers of ABCA1 expression in macrophages. Then total cell post-nuclear lysate (40ug protein) was separated by SDS-PAGE and detected using a 1:1000 dilution of NB 400-105 affinity purified Lot G incubated for 1 hour at room temperature (Lane A). Although there are lower molecular weight bands on the blot, the ABCA1 signal is excellent and gives the expected 3 bands. It is not known why ABCA1 runs as three bands, but it has been found to do so by many researchers. It is probably due to protein modifications such as glycosylation. The antibody was also tested against ABCA1 transiently expressed in 293 cells as an independent test with excellent results.

NOTE: An important factor in detecting ABCA1 is in the cell type used. ABCA1 is expressed in very low levels in most cell types. Therefore, ABCA1 expression needs to be induced by using 22-hydroxycholesterol and 9-cis-retinoic acid as ligands for the transcription factor LXR.

1. Without heating at all (leave at room temp for about 15 to 20 minutes with Beta-mercaptoethanol), load 40 ug post-nuclear lysates* to 7.5% or 4-15% Tris-HCL SDS gel (Bio-RAD) in sample buffer. Do NOT boil the samples. (NP-40 will not interfere with the running of the protein on SDS-PAGE.)
2. Transfer to nitrocellulose membrane at 100V 1hr or 30V overnight.
3. Block membrane in 5% milk in TBS-T for at least 1 hr. Wash with TBS-T 5 minutes.
4. Blot with anti-ABCA1 antibody in 3% milk in TBS-T for 1 hour.
5. Wash with TBS-T 3 times, 10 minutes each.
6. Blot with donkey anti-rabbit secondary; antibody (Amersham) at 1:2000 in 3% milk in TBS-T for 1 hour.
7. Wash with TBS-T 3 times, 10 minutes each.
8. Detect with chemiluminescent reagent (Pierce).
9. Expose to X-ray film, 30 sec., 1 min., and 3 min. TBS-T: Tris-buffered-saline with Tween-20

See also the specific references mentioned in the datasheet. *Post-nuclear lysate is the result of sonication or dounce homogenization of lysate, centrifugation at low-speed, and the removal of nuclei. The resulting supernatant is called post-nuclear and contains cytosolic and membrane proteins without any of the nuclear components.

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 C.
- 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 C 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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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 guarantee, please visit www.novusbio.com/guarantee.

