Product Datasheet

ABCA1 Antibody
NB400-105

Unit Size: 0.1 ml

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

Reviews: 6  Publications: 269

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## Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.05% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>PBS</td>
</tr>
<tr>
<td>Target Molecular Weight</td>
<td>220 kDa</td>
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## Product Description

<table>
<thead>
<tr>
<th>Feature</th>
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<tbody>
<tr>
<td>Host</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Gene ID</td>
<td>19</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>ABCA1</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat, Porcine, Canine, Chicken, Hamster, Mustelid</td>
</tr>
<tr>
<td>Reactivity Notes</td>
<td>Canine reactivity reported in scientific literature (PMID: 24612239)</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Partial peptide sequence (within residues 1100-1300) of human ABCA1 [UniProt# O95477]. Actual immunogen sequence is proprietary information.</td>
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## Product Application Details

<table>
<thead>
<tr>
<th>Feature</th>
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<tbody>
<tr>
<td>Applications</td>
<td>Western Blot, Simple Western, Chromatin Immunoprecipitation, ELISA, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Gel Supershift Assay</td>
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<tr>
<td>Recommended Dilutions</td>
<td>Western Blot 1:500, Simple Western 1:50, Chromatin Immunoprecipitation, Flow Cytometry 1:400, ELISA, Immunohistochemistry 1:200, Immunocytochemistry/Immunofluorescence 1:100, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen, Gel Supershift Assay</td>
</tr>
<tr>
<td>Application Notes</td>
<td>This ABCA1 antibody has been used for Flow Cytometry and ICC (PMID: 21501868), IP (PMID: 21846716; 21106522). Use in Gel supper shift assay reported in scientific literature (PMID 15684432). In Western blot a band representing ABCA1 is observed at approx. 220 kDa. Additional non-specific bands are seen at lower molecular weights, but do not interfere with the ABCA1 signal. Chromatin Immunoprecipitation and ELISA were also reported in scientific literature. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Charge. 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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</tbody>
</table>
Western Blot: ABCA1 Antibody [NB400-105] - Western Blot analysis of ABCA1 in total cell lysates of RAW264.9 cells treated with vehicle (-) or 9-cisretinoic acid and 22Rhydroxycholesterol (+). Samples used for this testing were 40 ug of total cell post-nuclear lysate from each group.

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.

Immunocytochemistry/Immunofluorescence: ABCA1 Antibody [NB400-105] - Untreated HepG2 cells were grown to 60% confluency, and serum starved for 24 hours prior to being fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-ABCA1 at 5.0ug/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: ABCA1 Antibody [NB400-105] - Detection of ABCA1 in human prostate epithelium showing luminal and membrane staining.
Immunocytochemistry/Immunofluorescence: ABCA1 Antibody [NB400-105] - HepG2 cells were grown to 60% confluency, serum starved for 24 hours, and then treated with 1uM TO9 for 24 hours prior to being fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-ABCA1 at 5.0ug/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.

Flow (Cell Surface): ABCA1 Antibody [NB400-105] - RAW 246.7 cells were either untreated (A) or serum starved for 24 hours, and then treated with 1uM TO9 for 24 hours (B). A surface stain was performed with ABCA1 Antibody NB400-105 (blue) and a matched isotype control NB810-56910 (orange). Cells were incubated in an antibody dilution of 5 ug/mL for 20 minutes at room temperature, followed by Goat Anti-Rabbit Dylight 550-conjugated antibody (SA5-10033, Thermo Fisher).

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-cis-retinoic acid and 22R-hydroxycholesterol.

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.

### Publications

Zinkhan EK, Yu B, Schlegel A. Prenatal Exposure to a Maternal High Fat Diet Increases Hepatic Cholesterol Accumulation in Intrauterine Growth Restricted Rats in Part Through MicroRNA-122 Inhibition of Cyp7a1 Front Physiol 43249 [PMID: 29896121] (WB, Rat)

Feng T, Liu P, Wang X et al. SIRT1 activator E1231 protects from experimental atherosclerosis and lowers plasma cholesterol and triglycerides by enhancing ABCA1 expression Atherosclerosis Apr 30 2018 12:00AM [PMID: 29787963] (WB, Mouse)


Widenmaier SB, Snyder NA, Nguyen TB et al. NRF1 Is an ER Membrane Sensor that Is Central to Cholesterol Homeostasis Cell. 2017 Nov 16 [PMID: 29149604] (WB)


Liang Y, Li X, Zhang Y et al. Induced Pluripotent Stem Cells-Derived Mesenchymal Stem Cells Attenuate Cigarette Smoke-Induced Cardiac Remodeling and Dysfunction Front Pharmacol 2017 Aug 14 [PMID: 28804458] (WB, Rat)


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. Blot with donkey anti-rabbit secondary; antibody (Amersham) at 1:2000 in 3% milk in TBS-T for 1 hour.

9. Detect with chemiluminescent reagent (Pierce).

10. 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.
Immunohistochemistry-Paraffin Protocol for ABCA1 Antibody (NB400-105)

Immunohistochemistry-Formalin Fixed Paraffin Embedded 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 1-1.5 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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