# **Product Datasheet**

# Apolipoprotein A-I/ApoA1 Antibody - BSA Free NBP2-52979

Unit Size: 0.1 mg

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

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#### NBP2-52979

Apolipoprotein A-I/ApoA1 Antibody - BSA Free

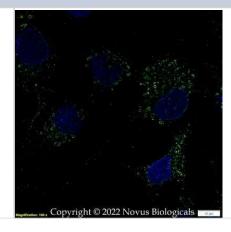
<b>Product Information</b>	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
<b>Product Description</b>	
Host	Rahhit

Host	Rabbit
Gene ID	335
Gene Symbol	APOA1
Species	Human, Mouse
Immunogen	Partial recombinant human ApoA1 protein (amino acids 25-267) [UniProt P02647]
Product Application Details	

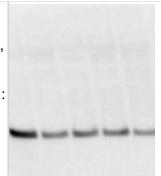
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 0.5 ug/ml - 1.0 ug/ml, Flow Cytometry 1 ug/million cells, Immunohistochemistry 1:400 - 1:1000, Immunocytochemistry/ Immunofluorescence 5 ug/ml, Immunohistochemistry-Paraffin 1:400 - 1:1000

# **Images**

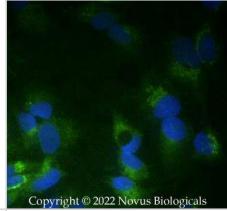
Immunocytochemistry/Immunofluorescence: Apolipoprotein A-I/ApoA1 Antibody - BSA Free [NBP2-52979] - HepG2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with Apolipoprotein A-1/ApoA1 Antibody (NBP2-52979) at 1ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



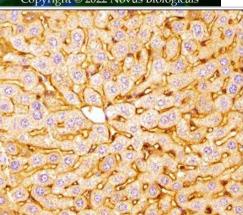
Western Blot: Apolipoprotein A-I/ApoA1 Antibody [NBP2-52979] - Western blot analysis using Apolipoprotein A-I/ApoA1 antibody. Harvested from mouse, separated on a 4-12 gradient gel by SDS-PAGE, transferred to PVDF membrane and blocked in 3% BSA/TBST. Probed with 1:8000 anti-apoA1 in 1%BSA/TBST, and detect edwith an anti-rabbit HRP secondary antibody using chemiluminescence. WB dilution 1:8000, block before incubation overnight. Image from verified customer review.



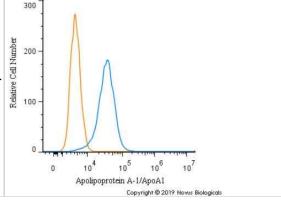
Immunocytochemistry/Immunofluorescence: Apolipoprotein A-I/ApoA1 Antibody - BSA Free [NBP2-52979] - HepG2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with Apolipoprotein A-1/ApoA1 Antibody conjugated to FITC (NBP2-52979F) at 5ug/ml for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



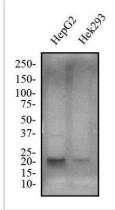
Immunohistochemistry-Paraffin: Apolipoprotein A-I/ApoA1 Antibody [NBP2-52979] - IHC analysis of a formalin fixed and paraffin embedded tissue section of mouse liver using APOA1 antibody at 1:400 dilution. The antibody generated a weak/mild cytoplasmic signal in hepatocytes with a very strong positivity near the hepatocyte membranes as well as in the inter-cellular spaces.



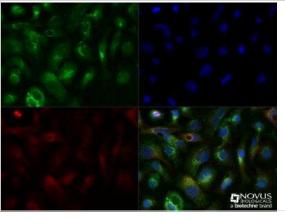
Flow Cytometry: Apolipoprotein A-I/ApoA1 Antibody [NBP2-52979] - An intracellular stain was performed on HepG2 cells with Apolipoprotein A-1/ApoA1 Antibody NBP2-52979 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, .



Western Blot: Apolipoprotein A-I/ApoA1 Antibody [NBP2-52979] - Total protein from HepG2 and Hek293 cells was separated on a 4-15% gradient gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 1.0 ug/ml anti-APOA1 in 1% milk, and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.



Immunocytochemistry/Immunofluorescence: Apolipoprotein A-I/ApoA1 Antibody [NBP2-52979] - HepG2 cells were 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-APOA1 at a 1:200 dilution 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.





#### **Procedures**

#### Western Blot protocol for Apolipoprotein A-I/ApoA1 Antibody (NBP2-52979)

Apolipoprotein A-I/ApoA1 Antibody:

Western Blot Protocol

- 1. Perform SDS-PAGE on samples to be analyzed, loading 25 ug of total protein per lane.
- 2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
- 3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
- 4. Rinse the blot.
- 5. Block the membrane using standard blocking buffer for at least 1 hour.
- 6. Wash the membrane in wash buffer three times for 10 minutes each.
- 7. Dilute anti-ApoA1 primary antibody in blocking buffer and incubate 1 hour at room temperature.
- 8. Wash the membrane in wash buffer three times for 10 minutes each.
- 9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
- 10. Wash the blot in wash buffer 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 manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

## Immunohistochemistry-Paraffin protocol for Apolipoprotein A-I/ApoA1 Antibody (NBP2-52979)

Apolipoprotein A-I/ApoA1 Antibody:

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.

#### Staining:

- 1. Wash sections in deionized water three times for 5 minutes each.
- 2. Wash sections in wash buffer for 5 minutes.
- 3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
- 7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
- 8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
- 9. Wash sections three times in wash buffer for 5 minutes each.
- 10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
- 11. As soon as the sections develop, immerse slides in deionized water.
- 12. Counterstain sections in hematoxylin.
- 13. Wash sections in deionized water two times for 5 minutes each.
- 14. Dehydrate sections.
- 15. Mount coverslips.



# Immunocytochemistry/Immunofluorescence protocol for Apolipoprotein A-I/ApoA1 Antibody (NBP2-52979) Apolipoprotein A-I/ApoA1 Antibody:

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

- 1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
- 2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
- 3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
- 4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
- 5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
- 6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
- 7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
- 8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
- 9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

\*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.





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# **Products Related to NBP2-52979**

HAF008 Goat anti-Rabbit IgG Secondary Antibody [HRP]

NB7160 Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]

NBP2-24891 Rabbit IgG Isotype Control

NBP2-34869-100ug Recombinant Human Apolipoprotein A-I/ApoA1 Protein

#### 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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