

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**

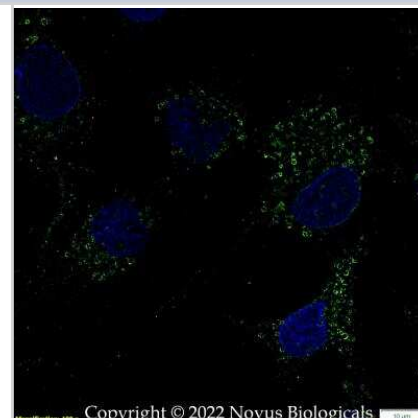
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Description	Novus Biologicals Rabbit Apolipoprotein A-I/ApoA1 Antibody - BSA Free (NBP2-52979) is a polyclonal antibody validated for use in IHC, WB, Flow and ICC/IF. All Novus Biologicals antibodies are covered by our 100% guarantee.
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	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry
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

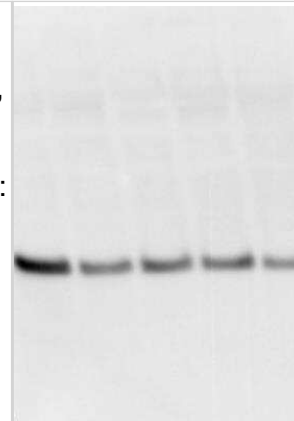
Images

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.

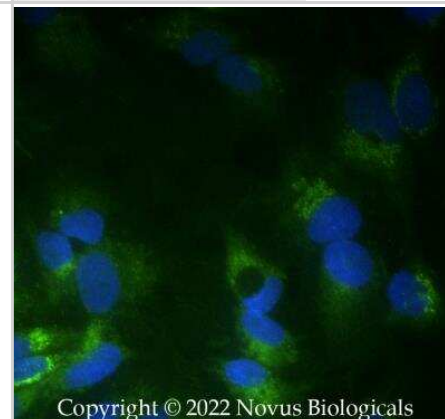


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Western Blot: Apolipoprotein A-I/ApoA1 Antibody [NBP2-52979] - Western blot analysis using Apolipoprotein A-I/ApoA1 antibody. Harvested from mouse, separated on a 4-12 gradient gel by SDS-PAGE, transferred to PVDF membrane and blocked in 3% BSA/TBST.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

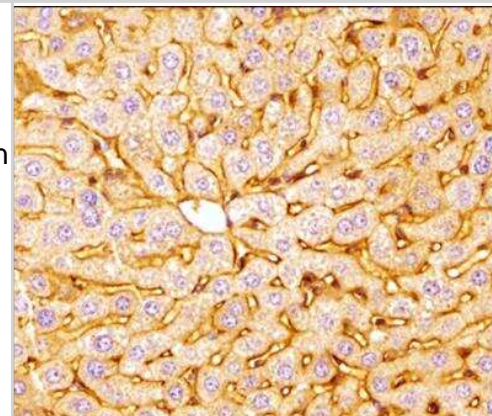


Immunocytochemistry/Immunofluorescence: Apolipoprotein A-I/ApoA1 Antibody - BSA Free [NBP2-52979] - HepG2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with Apolipoprotein A-1/ApoA1 Antibody conjugated to 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.

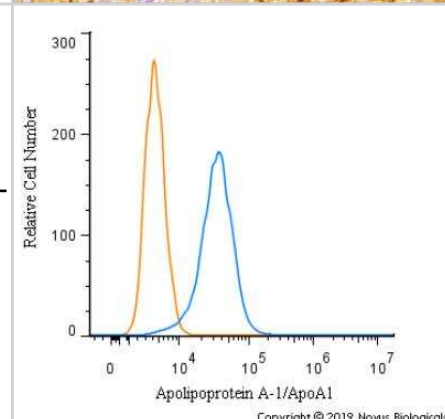


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Immunohistochemistry-Paraffin: Apolipoprotein A-I/ApoA1 Antibody [NBP2-52979] - IHC analysis of a formalin fixed and paraffin embedded tissue section of mouse liver using APOA1 antibody at 1:400 dilution. The antibody generated a weak/mild cytoplasmic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

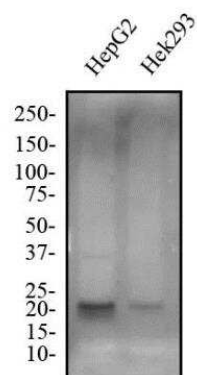


Flow Cytometry: Apolipoprotein A-I/ApoA1 Antibody [NBP2-52979] - An intracellular stain was performed on HepG2 cells with Apolipoprotein A-1/ApoA1 Antibody NBP2-52979 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated 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, .

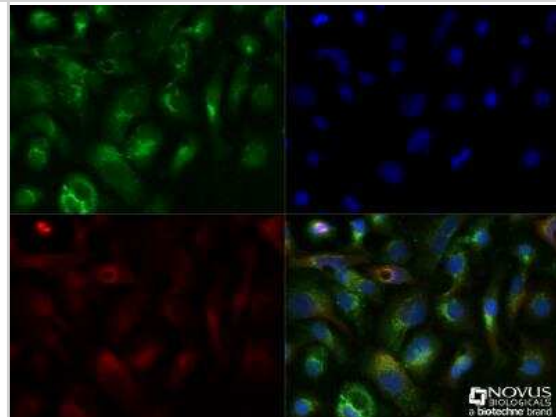


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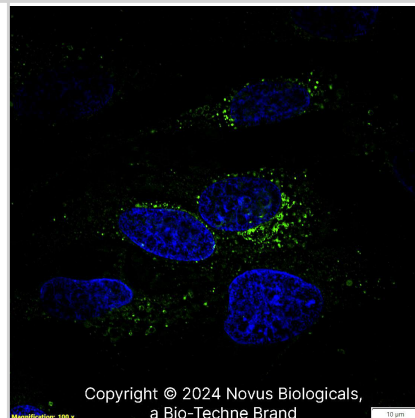
Western Blot: Apolipoprotein A-I/ApoA1 Antibody [NBP2-52979] - Total protein from HepG2 and Hek293 cells was separated on a 4-15% gradient gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 1.0 ug/ml anti-APOA1 in 1% milk, and detected with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



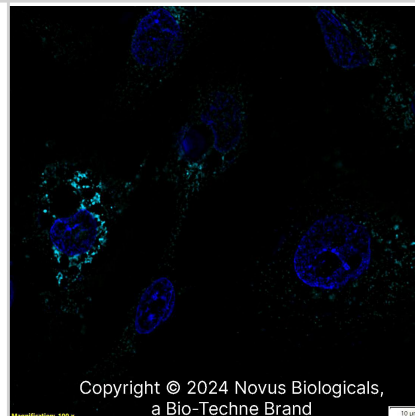
Immunocytochemistry/Immunofluorescence: Apolipoprotein A-I/ApoA1 Antibody [NBP2-52979] - HepG2 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-APOA1 at a 1:200 dilution overnight at 4C and detected 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.



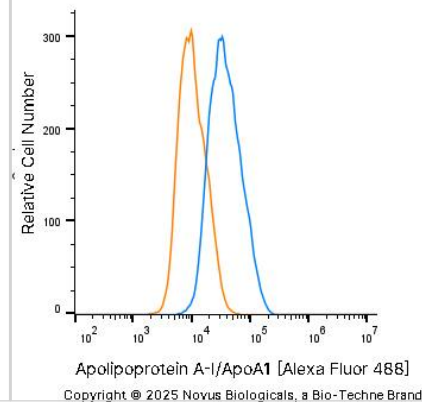
Apolipoprotein A-I/ApoA1 was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Rabbit anti-Apolipoprotein A-I/ApoA1 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NBP2-52979AF488) (green) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (dark blue). Cells were imaged using a 100X objective and digitally deconvolved.



Apolipoprotein A-I/ApoA1 was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Rabbit anti-Apolipoprotein A-I/ApoA1 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP2-52979AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (dark blue). Cells were imaged using a 100X objective and digitally deconvolved.



An intracellular stain was performed on HepG2 human hepatocellular carcinoma cell line with Rabbit anti-Apolipoprotein A-I/ApoA1 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 488 (Catalog # NBP2-52979AF488, blue histogram) or matched control antibody (Catalog # NBP2-24982, orange histogram) at 10 µg/mL for 30 minutes at RT.



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 1:25,000 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.



Flow (Intracellular) Protocol for Apolipoprotein A-I/ApoA1 Antibody (NBP2-52979)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2×10^5 and 1×10^6 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 μ L 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 1×10^6 cells/mL in staining buffer.
5. Aliquot out 100 μ L 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. 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 μ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 μ L of a permeabilization buffer to every 1×10^6 cells present in the sample. Mix well and incubate at room temperature for 10 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 10-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 μ L of staining buffer + 0.1% permeabilizer.
6. Add appropriate amounts of each antibody (eg. 1 test or 1 μ g per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes. 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 μ L for wells) and centrifuge 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 μ L for wells) and centrifuge at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 μ L per sample) and proceed with analysis on your flow cytometer.





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Products Related to NBP2-52979

NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
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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