ApoA1 Monoclonal Antibody (311)

Catalog Number: MIA1402

**Details**

- **Size**: 1 mg
- **Host/Isotype**: Mouse / IgG1
- **Class**: Monoclonal
- **Type**: Antibody
- **Clone**: 311
- **Immunogen**: Purified human plasma Apo A-1
- **Conjugate**: Unconjugated
- **Form**: Liquid
- **Concentration**: 5 mg/mL
- **Purification**: Protein A
- **Storage buffer**: PBS, pH 7.4
- **Contains**: 0.09% sodium azide
- **Storage Conditions**: Maintain refrigerated at 2-8°C for up to 6 months. For long term storage store at -20°C

**Species Reactivity**

- **Species reactivity**: Human, Mouse
- **Published species**: Human

**Tested Applications**

- **ELISA (ELISA)**: 1 µg/mL
- **Immunohistochemistry (Paraffin)** (IHC (P)): 1:100
- **Immunoprecipitation (IP)**: Assay-dependent
- **Radioimmune Assays (RIA)**: Assay-dependent
- **Western Blot (WB)**: 1:500-1:2,000
- **Immunocytochemistry (ICC/IF)**: 2 µg/mL

**Published Applications**

- **Functional Assay (FN)**: See 1 publications below
- **In vitro Assay (IV)**: See 1 publications below

**Product specific information**

By sandwich ELISA, MIA1402 can be used as a detection antibody with Product # 710263 or # 701239 as a coating antibody, to generate a matched pair. Using these matched pairs, recombinant human Apo A-1, but not recombinant mouse Apo A-1, was detected. MIA1402 can be used to detect Apo A-1 from serum samples. To increase sensitivity of sandwich ELISAs with MIA1402, a biotinylated detection antibody followed by Streptavidin-HRP is recommended. By Western blot, MIA1402 detects recombinant human Apo A-1, but not recombinant mouse Apo A-1. MIA1402 may not be as successful for detecting endogenous Apo A-1 by Western blot. MIA1402 was formerly sold as a Seradyn product.

**Background/Target Information**

Apolipoprotein A-1 (apo A-1) in human plasma is a major protein component of high density lipoprotein (HDL). The molecular mass is 24 kDa. Apo A-1 is a cofactor for enzymes involved in the normal turnover of cholesterol and is produced in the liver. Plasma concentration is 270-380 mg/100 mL.

ApoA1 Antibody (MIA1402)

Antibody specificity was demonstrated by detection of relative expression of the target protein across patient samples owing to their inherent genetic constitution. Increased expression of Apolipoprotein A1 in serum from patients suffering from dyslipidemia was detected in ELISA using anti-Apolipoprotein A1 monoclonal antibody (311) (Product # MIA1402).

ApoA1 Antibody (MIA1402) in ICC/IF

Immunofluorescence analysis of Apolipoprotein A1 was performed 70% confluent log phase Hep G2 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 2% BSA for 1 hour at room temperature. The cells were labeled with Apolipoprotein A1 (311) Mouse Monoclonal Antibody (Product # MIA1402) at 2 µg/mL in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28177) a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold AntiFade Mountant with DAPI (Product # S36938). Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing cytoplasmic localization. Panel e shows the no primary antibody control. The images were captured at 60X magnification.

ApoA1 Antibody (MIA1402) in IHC (P)

Immunohistochemistry analysis of Apolipoprotein A-1 showing staining in the cytoplasm of paraffin-embedded human liver tissue (right) compared to a negative control without primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with a Apolipoprotein A-1 Mouse Monoclonal Antibody (Product # MIA1402) diluted in 3% BSA-PBS at a dilution of 1:100 for 1 hour at 37ºC in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.

ApoA1 Antibody (MIA1402) in WB

Fig 1: Western blot analysis was performed on tissue extract (30 µg lysate) of Mouse Liver (Lane 1). Fig 2: Likewise, Western blot analysis was performed on samples of 3 µL serum (Lane 1), 5 µL serum (Lane 2), 5 µL plasma (Lane 3), all of which were obtained from a 50 year old male Alzheimer's patient. The blots were probed with Anti-Apolipoprotein A1 Mouse Monoclonal Antibody (Product # MIA1402, 1:500-1:2000 dilution) and detected by chemiluminescence Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.4 µg/mL, 1:2500 dilution). A 28 kDa band corresponding to Apolipoprotein A-1 was observed across the tissues and samples tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 12 % Bis-Tris gel (Product # NP0342BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).

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ApoA1 Antibody (MIA1402) in WB

Western blot analysis of Apolipoprotein A-1 was performed by loading the indicated amounts of a recombinant human Apo A-1-hlgG1Fc fusion protein (Product # 10686-H02H-50) or a recombinant mouse Apo A-1-hlgG1Fc fusion protein (Product # 50918-M02H-50), and 10 µL of PageRuler Prestained Protein Ladder (Product # 26616) per well onto a Novex® 4-20% Tris-Glycine polyacrylamide gel (Product # WT4202BOX). Proteins were transferred to a Nitrocellulose Membrane (Product # 38014) using the G2 Fast Blotter (Product # 62288), and blocked with 5% milk in TBST for at least 1 hour at room temperature. Apo A-1 was detected at ~50 kDa using an Apolipoprotein A-1 monoclonal antibody (Product # MIA1402) at a dilution of 1:1000 in blocking buffer overnight at 4°C on a rocking platform, followed by an HRP-conjugated goat-anti-mouse IgG Fc-specific secondary antibody (Product # 31439) at a dilution of 1:40,000 for at least 30 minutes at room temperature. Chemiluminescent detection was performed using SuperSignal West Dura (Product # 34075).

ApoA1 Antibody (MIA1402) in ELISA

Sandwich ELISA of Apolipoprotein A-1 was performed by coating wells of a 96-well plate with 100 µL of an Apo A-1 Recombinant Rabbit Polyclonal Antibody (Product # 710263) at a concentration of 1 µg/mL in carbonate/bicarbonate buffer (Product # 28382) overnight at 4°C. Wells were blocked with 150 µL of Blocking Buffer (TBS) Blocking Buffer (Product # 37543) for 30 minutes, and 80 µL of recombinant human Apo A-1 (Product # 10686-H02H-50) standards ranging from 1.6-1000 ng/mL (A) or 100 µL of diluted normal human serum or diluted serum from a patient with dyslipidemia (B) were incubated for 1 hour at room temperature. The plate was washed with 1X TBST (Product # 28360), and 100 µL per well of an Apo A-1 Mouse Monoclonal Antibody (Product # MIA1402) was added to each well for 1 hour at room temperature. The plate was washed, and 100 µL per well of a biotinylated Rabbit anti-Mouse IgG Superclonal secondary antibody (Product # A27024) was incubated for 1 hour, followed by Streptavidin-HRP (Product # 21126) for 30 minutes. Detection was performed using 1-Step Ultra TMB Substrate (Product # 34028), followed by Stop Solution (Product # N600). Absorbances were read on a spectrophotometer at 450-550 nm.

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ApoA1 Antibody (MIA1402) in ELISA

Sandwich ELISA of Apolipoprotein A-1 was performed by coating wells of a 96-well plate with 100 µL of an Apo A-1 rabbit monoclonal antibody (Product # 701239) diluted to a concentration of 1 µg/mL in carbonate/bicarbonate buffer (Product # 28382) overnight at 4C. Wells were blocked with 150 µL of StartingBlock T20 (TBS) Blocking Buffer (Product # 37543) for 30 minutes, and 80 µL of recombinant human Apo A-1 (Product # 10686-H02H-50) or recombinant mouse Apo A-1 (Product # 50918-M02H-50) was added to the plate at concentrations ranging from 1.6-1000 ng/mL and incubated for 1 hour at room temperature. The plate was washed, and 100 µL per well of an HRP-conjugated rabbit anti-mouse IgG cross-adsorbed secondary antibody (Product # 31452) was incubated for 30 minutes at room temperature. Detection was performed using 1-Step Ultra TMB Substrate (Product # 34028), followed by Stop Solution (Product # N600). Absorbances were read on a spectrophotometer at 450-550 nm.
### PubMed References For ApoA1 Monoclonal Antibody (311)

#### 1 Functional Assay References

<table>
<thead>
<tr>
<th>Species / Dilution</th>
<th>Summary</th>
</tr>
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<tbody>
<tr>
<td>Human / Not Cited</td>
<td>MIA1402 was used in Functional assays to investigate the relationship between genetic angiopoietin-like 3 polymorphism and Cholesterol uptake capacity in patients with cardiovascular disease.</td>
</tr>
</tbody>
</table>

*Journal of clinical laboratory analysis* (Dec 2021; 35: )

"Association of ANGPTL3 polymorphisms with high-density lipoprotein cholesterol uptake capacity in patients with cardiovascular disease."


PubMed Article URL: [http://dx.doi.org/10.1002/jcla.23980](http://dx.doi.org/10.1002/jcla.23980)

#### 1 In vitro Assay References

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</tr>
</tbody>
</table>

*Journal of clinical laboratory analysis* (Jun 2021; 35: )

"Serum HDL cholesterol uptake capacity in subjects from the MASHAD cohort study: Its value in determining the risk of cardiovascular endpoints."


PubMed Article URL: [http://dx.doi.org/10.1002/jcla.23770](http://dx.doi.org/10.1002/jcla.23770)