Human Apolipoprotein A1

ELISA Kit

Enzyme Immunoassay kit for the quantification of Apolipoprotein A1 in Human plasma and serum samples.

Catalog number: ARG81097

distributed in the US/Canada by:
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INTRODUCTION

Apolipoprotein A-I is the major protein component of high density lipoprotein (HDL) in plasma. The protein promotes cholesterol efflux from tissues to the liver for excretion, and it is a cofactor for lecithin cholesterolacyltransferase (LCAT) which is responsible for the formation of most plasma cholesteryl esters. This gene is closely linked with two other apolipoprotein genes on chromosome 11. Defects in this gene are associated with HDL deficiencies, including Tangier disease, and with systemic non-neuropathic amyloidosis. [provided by RefSeq, Jul 2008]

Apolipoprotein A1 participates in the reverse transport of cholesterol from tissues to the liver for excretion by promoting cholesterol efflux from tissues and by acting as a cofactor for the lecithin cholesterol acyltransferase (LCAT). As part of the SPAP complex, activates spermatozoa motility. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique for the detection of Human Apolipoprotein A1 in human plasma and serum samples. Human Apolipoprotein A1 will bind to the capture antibody coated on the microtiter plate. After appropriate washing steps, anti-Human Apolipoprotein A1 primary antibody binds to the captured protein. Following a washing to remove unbound substances, secondary antibody conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Apolipoprotein A1 bound in the initial step. The color development is stopped

by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Apolipoprotein A1 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 X 8 strips	4°C
Standard	1 vial (lyophilized)	4°C, store at -80 °C after reconstitution
Anti-Apolipoprotein A1 Primary Antibody	1 vial (lyophilized)	4°C
HRP-conjugated Secondary antibody	1 vial	4°C
10X Diluent	30 ml	
10X Wash buffer	50 ml	4°C
TMB substrate	10 ml (ready-to-use)	4°C (Protect from light)

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 1N H_2SO_4 or 1N HCl
- TBS buffer: 0.1M Tris, 0.15M NaCl, pH 7.4
- Blocking buffer (BB): 3% BSA (w/v) in TBS
- Pipettes and pipette tips

- Deionized or distilled water
- Automated microplate washer (optional)
- Orbital shaker

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reconstituted standards and primary antibody can be stored at -80°C for later use and avoid repeat freeze-thaw for more than once.
- Store the other unused kit components at 4°C at all times.
- Briefly spin down the Primary and Secondary Antibody before use.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Plasma: Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30

minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Diluent**: Dilute 30 ml of 10X Diluent concentrate with 270 ml of deionized water.
- **Standard**: Reconstitute standards by adding 1 ml of 1X Diluent directly to the vials and agitate gently to completely dissolve contents. This will result in a 5,000 ng/ml standard stock solution. Dilute the standard stock as the table at below with 1X Diluent and the 1X Diluent serves as zero standard (0 ng/ml).

  Dilution table for Human Apolipoprotein A1 standard preparation:

Apolipoprotein A1 Concentration (ng/ml)	Dilution
5,000	From the stock vial
2,000	600 µl Diluent + 400 µl (5,000 ng/ml)
1,000	500 µl Diluent + 500 µl (2,000 ng/ml)
500	500 µl Diluent + 500 µl (1,000 ng/ml)
200	600 µl Diluent + 400 µl (500 ng/ml)
100	500 µl Diluent + 500 µl (200 ng/ml)
50	500 µl Diluent + 500 µl (100 ng/ml)
20	600 µl Diluent + 400 µl (50 ng/ml)
10	500 µl Diluent + 500 µl (20 ng/ml)
5	500 µl Diluent + 500 µl (10 ng/ml)
0	500 µl Diluent as Zero point to determine background

  Dilutions for the standard curve and zero standard must be made and applied to the plate immediately.

- **1X Wash buffer**: Dilute 50 ml of 10X wash buffer concentrate with 450 ml

of deionized water.

- **Primary Antibody:** Briefly centrifuge vial before opening. Reconstitute primary antibody by adding 10 ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents.
- **Secondary Antibody:** Briefly centrifuge vial before opening. Dilute 2 µl of conjugated secondary antibody in 10ml of blocking buffer and agitate gently to mix.
- **Sample:** If the measuring absorbance of samples is higher than the highest standard, dilute the samples with 1x Diluent before assay and assay again. A 1:40,000 – 80,000 dilution for normal human plasma is suggested for best results.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples and zero controls into appropriate wells. Shake plate at 300 rpm for 30 minutes at RT.
3. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl of working Primary Antibody into each well. Shake plate at 300

rpm for 30 minutes at RT.

5. Wash as according to step 3.
6. Add 100 µl of working HRP-conjugated secondary Antibody into each well. Shake plate at 300 rpm for 30 minutes at RT.
7. Wash as according to step 3.
8. Add 100 µl of TMB substrate to each well. Incubate for 1-5 minutes at RT in dark. Substrate will change from colorless to different strengths of blue.
9. Add 50 µl of 1N H_2SO_4 or HCl to each well. The color of the solution should change from blue to yellow. Mix thoroughly by gently shaking the plate.
10. Read the OD with a microplate reader at 450 nm immediately.

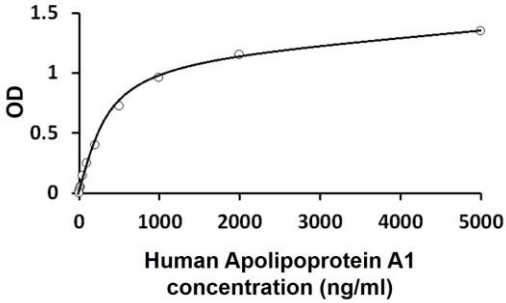
CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

6. EXPECTED VALUES: ApoA1 is present in human plasma and serum at a concentration of 0.75-1.6 mg/ml in adult males, 0.8-1.75 mg/ml in adult females, 0.38-1.06 mg/ml in newborns, and 0.6-1.67 mg/ml in children. The ratio of ApoA1/ApoB ranges from 0.85-2.24 in males and 0.76-3.23 in females.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

Assay range: 0 – 5000 ng/ml

Minimum Detectable Concentration: 4.9 ng/ml

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.