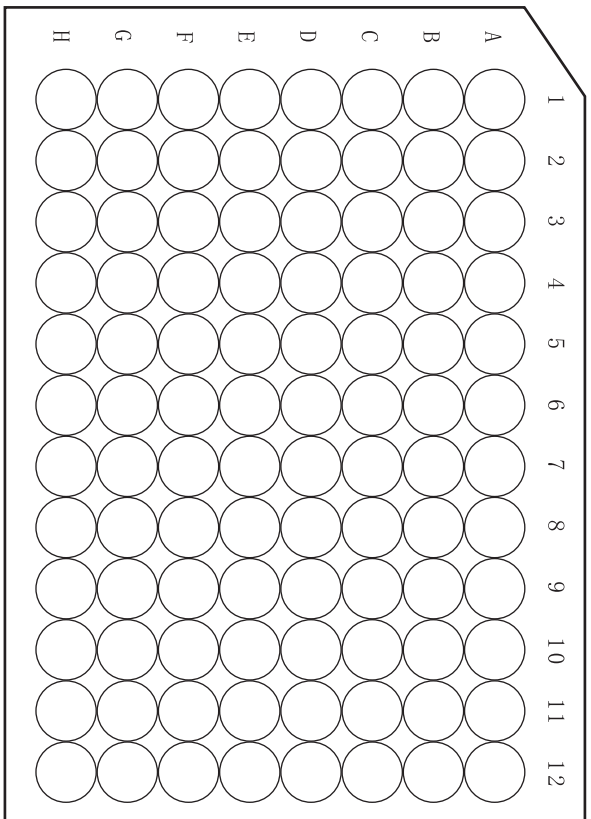

Human ApoA1 Immunoassay

Catalog Number:SEKH-0093

For the quantitative determination of human ApoA1 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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TABLE OF CONTENTS

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of ApoA1 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	92	104
	Range(%)	83—104	97—109
1:4	Average% of Expected	95	105
	Range(%)	87—110	95—110
1:8	Average% of Expected	95	106
	Range(%)	88—108	86—108
1:16	Average% of Expected	99	104
	Range(%)	92—106	91—117

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 1.5ng/mL.
SPECIFICITY: This assay recognizes both natural and recombinant human ApoA1. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
ApoE3		
Cubilin		
LDL R		
SR-AI		
SR-BI		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of ApoA1 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of ApoA1 in two matrices

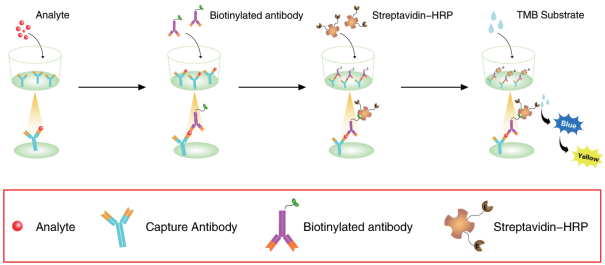
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	99	86—105
Cell culture supernatants	97	92—105

BACKGROUND

The apolipoproteins are a structurally-unrelated group of proteins that have some association with the transport of lipids in blood. Apolipoproteins, plus phospholipids, cholesterol and triglycerides, form spherical particles with a lipid/hydrophobic center and a (apolipo)protein coat. The apolipoprotein coat promotes aqueous solubility and serves as a ligand for lipoprotein receptors. HDL may contain apolipoproteins A, C, D, E, J, L and M, while LDL contains apolipoproteins B and E.
ApoAI and ApoA2 are major protein components of serum high-density lipoprotein (HDL) and are produced by the liver and small intestine. They are involved in reverse cholesterol transport from tissues to the liver. Polymorphisms of ApoA2 are associated with disorders of cholesterol and fatty acid metabolism.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for ApoA1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and anyApoA1 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for ApoA1is added to detect the captured ApoA1 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugatedStreptavidinis added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

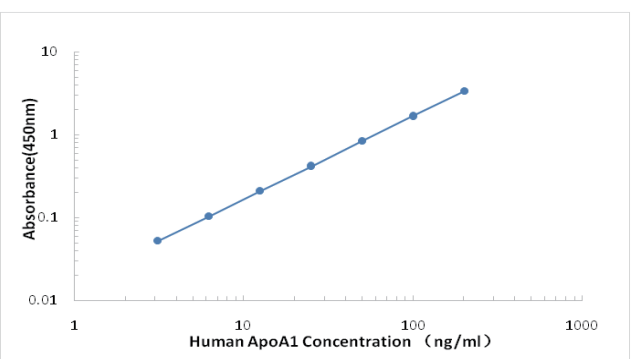
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the ApoA1 ELISA

Standared(ng/ml)	OD.	OD.	Average	Corrected
0	0.048	0.052	0.050	-
3.13	0.200	0.193	0.197	0.147
6.3	0.291	0.281	0.286	0.236
13	0.433	0.417	0.425	0.375
25	0.688	0.663	0.675	0.626
50	1.117	1.076	1.097	1.047
100	1.819	1.753	1.786	1.736
200	2.964	2.856	2.910	2.860



Representative standard curve for ApoA1 ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed.Wash three times before assay.

⇩

Add 100µl standard or samples to each well, incubate 120 minutes,at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human ApoA1 antibody to each well, incubate 60 minutes,at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes,at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 30 minutes,at room temperature(25±2℃).Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

Note: oscillatory reaction at room temperature 400r

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the ApoA1 concentrations versus the log of the O.D. and the best fit line can be determined by

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack.Reseal along entire edge of the zip-seal.May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,200ng/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

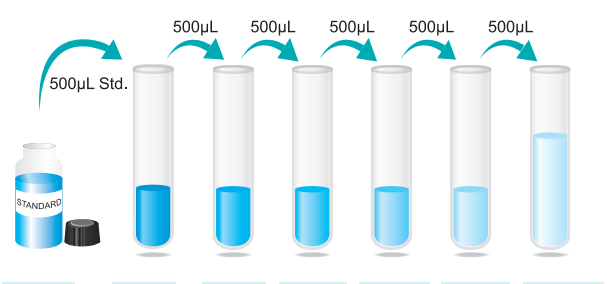
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000g(or 3000rpm). Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g(or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature(20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard\Sample - Reconstitute the Standard with 1mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 200ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500ul of sandard/sample dilution into the 100ng/ml tube ,and add 500ul stock solution of 200ng/ml into it to get the standard of 100ng/ml ,use the high standard to produce a 2-fold dilution series(below) . Mix each tube thoroughly and change pipette tips between each transfer.The200ng/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).**
   ***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**
4. **Working solution of Biotin-Conjugate anti-human IL-6 antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP:** Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**



Preparation of Apo A1 standard dilutions