Human Apolipoprotein E ELISA Kit (Rapid One-Step)

Enzyme Immunoassay for the quantification of Human Apolipoprotein E in serum, plasma (heparin, EDTA), saliva, urine, human milk and cell culture supernatants

Catalog number: ARG83332

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

The protein encoded by this gene is a major apoprotein of the chylomicron. It binds to a specific liver and peripheral cell receptor, and is essential for the normal catabolism of triglyceride-rich lipoprotein constituents. This gene maps to chromosome 19 in a cluster with the related apolipoprotein C1 and C2 genes. Mutations in this gene result in familial dysbetalipoproteinemia, or type III hyperlipoproteinemia (HLP III), in which increased plasma cholesterol and triglycerides are the consequence of impaired clearance of chylomicron and VLDL remnants. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Nov 2014]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for apolipoprotein E has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any apolipoprotein E present is bound by the immobilized antibody. Then Antibody conjugated specific for apolipoprotein E is added to each well and incubate. Following a washing to remove unbound substances, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of apolipoprotein E 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 E in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

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

NO	Component	Quantity	Storage information
C1	Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
C2	Standard (Lyophilized)	2 X 200 ng/vial	4°C
C3	Diluent buffer	30 ml (ready to use)	4°C
C4	Antibody conjugated	1 vial (ready to use)	4°C
C5	25X Wash buffer	20 ml	4°C
C6	TMB substrate	10 ml (ready to use)	4°C (Protect from light)
C7	STOP solution	10 ml (ready to use)	4°C
C8	Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- If crystals are observed in the 25X 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.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 1 min or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.
- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Avoid using reagents from different batches.
- 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.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. 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. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Saliva- Collect saliva using a collection device (e.g. Salivette), centrifuge 10,000 x g for 2 min at 4°C. Collect saliva and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles. The collection device should not have protein binding or filtering features.

Urine- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months.

Milk- Centrifuge samples 1500 x g for 15 min at 4°C. Collect the aqueous fraction and repeat three times. Filter the samples through a 0.2 µm filter. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months.

Note:

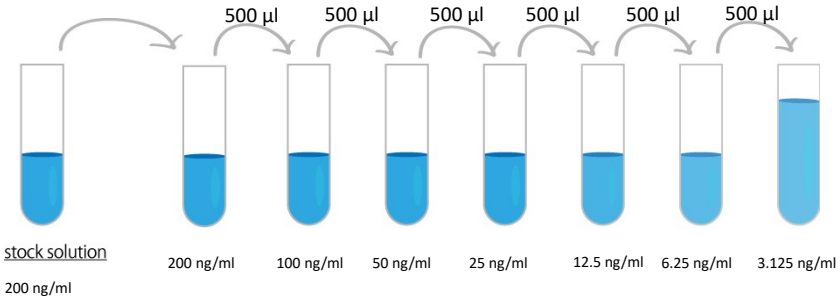
a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute **25X** Wash buffer (#C5) into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 25X Wash buffer + 1200 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- **Sample**: If the initial assay found samples contain apolipoprotein E higher than the highest standard, the samples can be diluted with diluent buffer (#C3) and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.
  (It is recommended to do pre-test to determine the suitable dilution factor).
- **Standards**: Reconstitute the standard (#C2) with **1 ml** diluent buffer (#C3)

to yield a stock concentration of **200 ng/ml.** Keep the buffer in the vail for at least **15 min at RT** to make sure the standard is dissolved completely before making serial dilutions. The diluent buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: **200 ng/ml, 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.125 ng/ml**. DO NOT reuse the reconstituted standard.



Dilute apolipoprotein E standard as according to the table below:

Standard	apolipoprotein E Conc.	µl of Standard diluent	µl of standard
S7	200 ng/ml	0	1000 (200 ng/ml Stock)
S6	100 ng/ml	500	500 (S7)
S5	50 ng/ml	500	500 (S6)
S4	25 ng/ml	500	500 (S5)
S3	12.5 ng/ml	500	500 (S4)
S2	6.25 ng/ml	500	500 (S3)
S1	3.125 ng/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 20 min 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 **50 µl** of standards, samples and zero controls (diluent buffer) into wells.
3. Add **50 µl** 1X Antibody conjugated into each well, gently tap the plate to mix well. Cover wells and incubate for **1 hour at Room Temperature**.
4. Aspirate each well and wash, repeating the process three times for a total **four washes**. Wash by filling each well with 1× Wash Buffer (**300 µl**) using a squirt bottle, manifold dispenser, or autowasher, keep the Wash Buffer in the wells for 30 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **90 µl** of TMB Reagent (#C6) to each well, gently tap the plate to mix well. Incubate for **15-25 minutes at Room Temperature** in dark.
6. Add **100 µl** of Stop Solution (#C7) to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at **450 nm immediately**. It is recommended read the absorbance within 3 min after adding STOP solution.

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. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

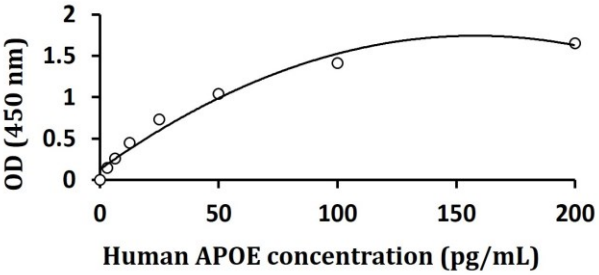
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

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

The minimum detectable dose (MDD) of Human Apolipoprotein E ranged from 3.12 ng/ml- 200 ng/ml. The mean MDD was 60 pg/ml.

Specificity

This assay recognizes natural and recombinant Human Apolipoprotein E.

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.