Human Apolipoprotein E ELISA Kit

Enzyme Immunoassay for the quantification of human Apolipoprotein E (ApoE) in human plasma, serum and biological fluids

Catalog number: ARG81211

distributed in the US/Canada by:
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For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Apolipoprotein E (ApoE) 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]

Apolipoprotein E (ApoE) mediates the binding, internalization, and catabolism of lipoprotein particles. It can serve as a ligand for the LDL (apo B/E) receptor and for the specific apo-E receptor (chylomicron remnant) of hepatic tissues. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for ApoE has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any ApoE present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for ApoE is added to each well and incubate. Following a washing to remove unbound substances, streptavidin 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 ApoE 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 ApoE 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.

Component	Quantity	Storage information
antibody-coated microplate	12 X 8 strips	4°C
Standard (10 µg/ml)	50 µl	-20°C
10X Wash Buffer	100 ml	4°C
Conjugated-ApoE Antibody concentrate	20 µl	4°C
HRP-Streptavidin concentrate	20 µl	4°C
Assay Diluent	50 ml	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: 620 nm as optional reference wave length)
- Pipettes and pipette tips
- Multichannel micropipette reservoir

- Deionized or distilled water
- 1X PBS containing 0.1% BSA.
- Microplate shaker.
- 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 4°C at all times.
- Upon receipt, the Standard should be aliquoted and stored at ≤ -20 °C to avoid repeated freeze-thaw cycles.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to 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.
- It is highly recommended that the standards and samples 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.

Serum- Use a serum separator tube (SST) and allow samples to clot for few minutes before centrifugation for 10 minutes at 1000 x g at 4°C. Collect serum and assay immediately or aliquot and store samples at -80°C up to 3 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant at 4°C. Centrifuge for 10 minutes at 1000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at-80°C up to 3 months. Avoid repeated freeze-thaw cycles.

Other Biological Fluids - Centrifuge samples for 10 minutes at 1000 g at 4°C. Assay immediately or aliquot and store samples at-80°C up to 3 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer, mix well. Storage at 2-8°C.
- 1X Conjugated-ApoE Antibody working solution: Dilute the antibody immediately before use; dilute the 1000X Conjugated-ApoE Antibody concentrate into Assay Diluent to yield 1X Conjugated antibody working solution. (E.g.: 5µl of the Conjugated-ApoE Antibody concentrate (1000X) + 5ml of Assay Diluent) Do not store diluted solutions.
- 1X HRP-Streptavidin working solution: Dilute the reagent immediately before use; dilute the 1000X HRP-Streptavidin concentrate into Assay

Diluent to yield 1X HRP-Streptavidin working solution. (E.g.: 5µl of the HRP-Streptavidin concentrate (1000X) + 5ml of Assay Diluent) Do not store diluted solutions.

- **Human ApoE standard:** Prepare a series dilution of Human ApoE standards with Assay Diluent. The Assay Diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

Standard No	Human ApoE (pg/ml)	Assay Diluent (µl)	Standards (µl)
S1	10000	1998	2 (10µg/ml stock)
S2	5000	500	500 µl (S1)
S3	2500	500	500 µl (S2)
S4	1250	500	500 µl (S3)
S5	625	500	500 µl (S4)
S6	312.5	500	500 µl (S5)
S7	156.25	500	500 µl (S6)
S0	0	500	0

Note: Dilutions for the standard must be made and applied to the plate immediately. S0 serves as background.

- **Sample**: Normal plasma or serum samples should be diluted at least 20,000 fold dilution or more with 0.1% BSA contained 1X PBS immediately before assay. If the assay found samples contain ApoE still higher than the highest standard, the samples can be further diluted with 0.1% BSA contained 1X PBS 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).

ASSAY PROCEDURE

Warm Substrate Solution to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Standards and samples should be assayed in duplicates.
2. Add 100 µl of the Standards and diluted samples into the appropriate wells. Incubate for at least 2 hours at 37°C or incubate overnight at 4°C on a microplate shaker.
3. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (250 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 Wash Buffer by aspirating, decanting or blotting
4. Add 100 µl of the 1:1000 diluted Conjugated-ApoE antibody working solution to each well, incubate for 1 hour at RT on a microplate shaker.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of the diluted HRP-Streptavidin working solution to all wells and incubate for 1 hour at RT on a microplate shaker.
7. Warm TMB substrate solution to RT before next wash step. Aspirate each well and wash as step 3. Proceed immediately to the next step.
8. Add 100 µl of TMB substrate solution into each well. Incubate for 2-30 mins at RT on microplate shaker. Avoid exposure to light.
   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Add 100 µl of Stop Solution to each well and shake lightly to ensure homogeneous mixing.
10. Read the OD with a microplate reader at 450nm immediately (optional: read at 620 nm as reference wave length).

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and 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.

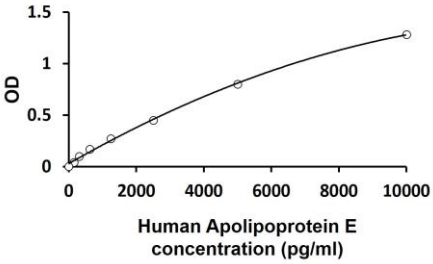
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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. 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

200 pg/ml

Assay Range

0 –10000 pg/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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