

Human Apolipoprotein E (Apo-E)ELISA Kit Catalog No. CSB-E09748h

(96 T)

This immunoassay kit allows for the in vitro quantitative determination of **human Apo-E** concentrations in **serum, plasma, Urine, Cell Culture Supernates and Tissue Homogenates**. **Expiration date six months from the date of manufacture**
FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Apolipoprotein E is a type of lipoprotein (a protein connected to a fat). Apolipoprotein E is abbreviated ApoE and the gene that encodes it is known as APOE. APOE is located on chromosome 19 in band 19q13.2. There are three isoforms (slightly different forms) of the ApoE lipoprotein, ApoE2, E3 and E4. They are encoded by slightly different versions, or alleles, of the APOE gene. Lipoproteins are responsible for carrying cholesterol and other fats through the bloodstream as little packages and are essential for the normal breakdown of these molecules. In particular, apolipoprotein E is a major component of specific lipoproteins called very low-density lipoproteins (VLDL). A major function of VLDLs is to remove excess cholesterol from the blood and carry it to the liver for processing. Maintaining normal levels of cholesterol is essential for the prevention of cardiovascular diseases, including heart attacks and strokes. APOE was initially recognized for its importance in lipoprotein metabolism and cardiovascular disease. More recently, it has been studied for its role in several biological processes not directly related to lipoprotein transport, including Alzheimer's disease (AD), immunoregulation, and cognition. Apolipoprotein E is also associated with several cardiovascular disorders.

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with an antibody specific to Apo-E. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for Apo-E and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain Apo-E, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of $450 \text{ nm} \pm 2 \text{ nm}$.

The concentration of Apo-E in the samples is then determined by comparing the O.D. of the samples to the standard curve.

DETECTION RANGE

1.56 ng/ml-100ng/ml. The standard curve concentrations used for the ELISA's were 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.12 ng/ml, 1.56 ng/ml.

SPECIFICITY

This assay recognizes human Apo-E. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of human Apo-E is typically less than 0.4ng/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent Quantity

Assay plate	1
Standard	2
Sample Diluent	2 x 20 ml
Biotin-antibody Diluent	1 x 10 ml
HRP-avidin Diluent	1 x 10 ml
Biotin-antibody	1 x 120 μ l
HRP-avidin	1 x 120 μ l
Wash Buffer	1 x 20 ml (25 \times concentrate)
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml

STORAGE

1. Unopened test kits should be stored at 2-8 $^{\circ}$ C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit, provided it is stored as prescribed above. Refer to the package label for the expiration date.
2. Opened test plate should be stored at 2-8 $^{\circ}$ C in the aluminum foil bag with desiccants to minimize exposure to damp air. The kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer.

2. **Standard** Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the **Standard** with 1.0 ml of **Sample Diluent**. This reconstitution produces a stock solution of 100 ng/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (100 ng/ml). The **Sample Diluent** serves as the zero standard (0 ng/ml). Prepare fresh for each assay. Use within 4 hours and discard after use.

3. **Biotin-antibody** Centrifuge the vial before opening. Dilute to the working concentration using **Biotin-antibody Diluent**(1:100), respectively.

4. **HRP-avidin** Centrifuge the vial before opening. Dilute to the working concentration using **HRP-avidin Diluent**(1:100), respectively.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.

Pipettes and pipette tips.

Deionized or distilled water.

Squirt bottle, manifold dispenser, or automated microplate washer.

An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

SAMPLE COLLECTION AND STORAGE

Serum Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Urine Collect urine using a metabolic cage. Remove any particulates by centrifugation for 15 minutes at 1000x g, 2 - 8°C and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

Cell Culture Supernates Remove particulates by centrifugation for 15 minutes at 1000 x g, 2 - 8°C and assay immediately or aliquot and store samples at -20° C or -80°C. Avoid repeated freeze-thaw cycles.

Tissue Homogenates 100mg tissue was rinsed with 1X PBS, homogenized in 1 mL of 1X PBS and stored overnight at -20° C. After two freeze-thaw cycles were performed to break the

cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. The supernate was assayed and removed immediately. Alternatively, aliquot and store samples at -20°C or -80. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.

1. Recommend to dilute the serum or plasma samples with Sample Diluent(1:1000) before test. The suggested 1000-fold dilution can be achieved by adding 5µl sample to 95µl of Sample Diluent. Complete the 1000-fold dilution by adding 5µl of this solution to 245µl of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.
2. Add 100µl of Standard, Blank, or Sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37°C.
3. Remove the liquid of each well, don't wash.
4. Add 100µl of **Biotin-antibody** working solution to each well. Incubate for 1 hour at 37°C. **Biotin-antibody** working solution may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.
5. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash: Fill each well with Wash Buffer (200µl) and let it stand for 2 minutes, then remove the liquid by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel. Complete removal of liquid at each step is essential to good performance.
6. Add 100µl of **HRP-avidin** working solution to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.
7. Repeat the aspiration and wash five times as step 5.
8. Add 90µl of **TMB Substrate** to each well. Incubate for 10-30 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
9. Add 50µl of **Stop Solution** to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Using the professional soft "Curve Exert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create 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 x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Apo-E concentrations versus the log of the O.D. and the best fit line can be determined by 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.

LIMITATIONS OF THE PROCEDURE

The kit should not be used beyond the expiration date on the kit label.

Do not mix or substitute reagents with those from other lots or sources.

It is important that the Standard Diluent selected for the standard curve be consistent with the samples being assayed.

If samples generate values higher than the highest standard, dilute the samples with the appropriate Standard Diluent and repeat the assay.

Any variation in Standard Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

Centrifuge vials before opening to collect contents.

When mixing or reconstituting protein solutions, always avoid foaming.

To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Substrate Solution should remain colorless or light blue until added to the plate. Keep Substrate Solution protected from light.

Substrate Solution should change from colorless or light blue to gradations of blue. Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.