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Apolipoprotein E

Human ELISA Kit

For the quantitative measurement of human Apolipoprotein E in plasma, serum, cerebrospinal fluid and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview	1
2. Protocol Summary	2
3. Precautions	3
4. Storage and Stability	3
5. Limitations	4
6. Materials Supplied	4
7. Materials Required, Not Supplied	5
8. Technical Hints	6
9. Reagent Preparation	7
10. Standard Preparation	10
11. Sample Preparation	11
12. Plate Preparation	13
13. Assay Procedure	14
14. Typical Data	16
15. Typical Sample Values	17
16. Assay Specificity	18
17. Species Reactivity	18
18. Troubleshooting	19
19. Notes	21
Technical Support	22

1. Overview

Human Apolipoprotein E (APOE) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Apolipoprotein E concentrations in human plasma, serum and cerebrospinal fluid (CSF) samples.

An Apolipoprotein E specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently an Apolipoprotein E specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of Apolipoprotein E captured in plate. The kit recognizes ApoE-2, ApoE-3, and ApoE-4 isoforms.

Apolipoprotein E is a 34 kDa polymorphic protein with 299 amino acids and occurs in all lipoprotein fractions in plasma. It is synthesized primarily by the liver and is a main apoprotein of the chylomicron. Apolipoprotein E is essential for the normal catabolism of triglyceride-rich lipoprotein constituents and cardiovascular diseases. Apolipoprotein E is also critical in several other important biological processes, including Alzheimer's disease, cognitive function, immunoregulation, cell signaling, and infectious diseases. There are three common isoforms of the protein: apoE-3 is normal; while apoE-2 and apoE-4 are dysfunctional. Apolipoprotein E deficiency causes type III hyperlipoproteinemia and premature atherosclerosis. Apolipoprotein E is a major genetic risk factor for late-onset familial Alzheimer's disease and for cognitive deficits associated with aging. Apolipoprotein E-4 enhances HIV-1 cell entry *in vitro* and the Apolipoprotein E $\epsilon 4/\epsilon 4$ genotype accelerates HIV disease progression.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add standard or sample to appropriate wells.

Incubate at room temperature.



Wash and add prepared biotin antibody to each well. Incubate at room temperature.



Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.



Add Chromogen Substrate to each well. Incubate at room temperature



Add Stop Solution to each well. Read immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Antibody, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Apolipoprotein E Microplate (12 x 8 wells)	96 wells	4°C
Apolipoprotein E Standard	2 vials	-20°C
10X Diluent M Concentrate	30 mL	4°C
Biotinylated human Apolipoprotein E Antibody	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Standard Diluent	2 mL	4°C
Sealing Tapes	3	N/A

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 μ L to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 6 tubes to prepare standard or sample dilutions.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare fresh reagents immediately prior to use.
- When diluting concentrates, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

9.1 1X Diluent M

Dilute the 10X Diluent M Concentrate 1:10 with reagent grade water. Mix gently and thoroughly.

Δ Note: Store for up to 1 month at 4°C.

9.2 1X Wash Buffer

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

9.3 1X Biotinylated Apolipoprotein E Detector Antibody

9.3.1 The stock Biotinylated Apolipoprotein E Antibody must be diluted with 1X Diluent M according to the label concentration to prepare 1X Biotinylated Apolipoprotein E Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated Apolipoprotein E Antibody.

9.3.2 Calculate the necessary amount of 1X Diluent M to dilute the Biotinylated Apolipoprotein E Antibody to prepare a 1X Biotinylated Apolipoprotein E Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

Number of Wells Strips	Number of Wells	(V _T) Total Volume of 1X Biotinylated Antibody (μL)
4	32	1,760
6	48	2,640
8	64	3,520
10	80	4,400
12	96	5,280

Δ Note: Any remaining solution should be frozen at -20°C.

Where:

C_S = Starting concentration (X) of stock Biotinylated Apolipoprotein E Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated Apolipoprotein E Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated Apolipoprotein E Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated Apolipoprotein E Antibody

V_D = Total volume of 1X Diluent M required to dilute (X) stock Biotinylated Apolipoprotein E Antibody to prepare 1X Biotinylated Apolipoprotein E solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells:

$$(C_F / C_S) \times V_T = V_A$$

Calculate the final volume of 1X Diluent M required to prepare the 1X Biotinylated Apolipoprotein E Antibody:

$$V_T - V_A = V_D$$

Example:

Δ Note: This example is for demonstration purposes only. Please remember to check your antibody vial for the actual concentration of antibody provided.

C_S = 30X Biotinylated Apolipoprotein E Antibody stock

C_F = 1X Biotinylated Apolipoprotein E Antibody solution for use in the assay procedure

V_T = 3,520 μ L (8 well strips or 64 wells)

$$(1X/30X) \times 3,520 \mu\text{L} = 117.3 \mu\text{L}$$

$$3,520 \mu\text{L} - 117.3 \mu\text{L} = 3,402.7 \mu\text{L}$$

V_A = 117.3 μ L total volume of (X) stock Biotinylated Apolipoprotein E Antibody required

V_D = 3,402.7 μ L total volume of 1X Diluent M required to dilute the 30X stock Biotinylated Antibody to prepare 1X Biotinylated Apolipoprotein E Antibody solution for assay procedures.

- 9.3.3 First spin the Biotinylated Apolipoprotein E Antibody vial to collect the contents at the bottom.
- 9.3.4 Add calculated amount V_A of stock Biotinylated Apolipoprotein E Antibody to the calculated amount V_D of 1X Assay Diluent M. Mix gently and thoroughly.

9.4 1X SP Conjugate

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent M.

Δ Note: Any remaining solution should be frozen at -20°C.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitution of the Apolipoprotein E Standard vial to prepare a 1 µg/mL **Stock Standard**.

- 10.1.1 First consult the Apolipoprotein E Standard vial to determine the mass of protein in the vial.
- 10.1.2 Calculate the appropriate volume of Standard Diluent to add when resuspending the Apolipoprotein E Standard vial to produce an 1 µg/mL dilution by using the following equation:

C_S = Starting mass of Apolipoprotein E Standard (see vial label)
(µg)

C_F = The 8 µg/mL Apolipoprotein E required concentration

V_D = Required volume of Standard Diluent for reconstitution (µL)

Calculate total required volume Standard Diluent for resuspension:

$$(C_S / C_F) \times 1,000 = V_D$$

Example:

Δ Note: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 0.17 µg of Apolipoprotein E Standard in vial

C_F = 1 µg/mL Apolipoprotein E final concentration

V_D = Required volume of Standard Diluent for reconstitution

$$(0.17 \mu\text{g} / 1 \mu\text{g/mL}) \times 1,000 = 170 \mu\text{L}$$

- 10.1.3 First briefly centrifuge the Apolipoprotein E Standard Vial to collect the contents on the bottom of the tube.
- 10.1.4 Reconstitute the Apolipoprotein E Standard vial by adding the appropriate calculated amount V_D of Standard diluent to the vial to generate the 1 $\mu\text{g/mL}$ **Stock Standard**. Mix gently and thoroughly.
- 10.2 Allow the reconstituted 1 $\mu\text{g/mL}$ Apolipoprotein E **Stock Standard** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- 10.3 Label eight tubes #1 – 8.
- 10.4 Prepare the 0.5 $\mu\text{g/mL}$ **Standard #1** by adding 120 μL of the reconstituted 1 $\mu\text{g/mL}$ **Stock Standard** to 120 μL of 1X Diluent M.
- 10.5 Add 120 μL of 1X Diluent M to tube #2 – 8.
- 10.6 To prepare **Standard #2**, add 120 μL of the **Standard #1** into tube #2 and mix gently.
- 10.7 To prepare **Standard #3**, add 120 μL of the **Standard #2** into tube #3 and mix gently.
- 10.8 Using the table below as a guide, prepare subsequent serial dilutions.
- 10.9 1X Diluent M serves as the zero standard (0 $\mu\text{g/mL}$).

Standard #	Volume to dilute (μL)	Volume Diluent M (μL)	Human Apolipoprotein E ($\mu\text{g/mL}$)
1	Step 10.4		0.5
2	120 μL Standard #1	120	0.25
3	120 μL Standard #2	120	0.125
4	120 μL Standard #3	120	0.063
5	120 μL Standard #4	120	0.031
6	120 μL Standard #5	120	0.016
7	120 μL Standard #6	120	0.008
8 (Blank)	N/A	120	0

11. Sample Preparation

11.1 Plasma:

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x g for 10 minutes. Dilute samples 1:1600 into 1X Diluent M and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as an anticoagulant.).

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x g for 10 minutes. Dilute samples 1:1600 into 1X Diluent M and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Cerebrospinal Fluid:

Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3,000 rpm for 10 minutes. Dilute samples 1:20 (or within the range of 1:2 – 1:100) into 1X Diluent M and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Δ Note: Applicable samples may also include biofluids, cell culture and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater <i>(for reference only; please follow the insert for specific dilution suggested)</i>	
100x	10000x
4 µl sample + 396 µl buffer (100X) = 100-fold dilution <i>Assuming the needed volume is less than or equal to 400 µl</i>	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution <i>Assuming the needed volume is less than or equal to 400 µl</i>
1000x	100000x
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution <i>Assuming the needed volume is less than or equal to 240 µl</i>	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution <i>Assuming the needed volume is less than or equal to 240 µl</i>

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
 - We recommend that you assay all standards, controls and samples in duplicate.
-
- 13.1** Prepare all reagents, working standards, and samples as directed in the previous sections. The assay is performed at room temperature (20-25°C).
 - 13.2** Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
 - 13.3** Add 50 µL of Apolipoprotein E Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
 - 13.4** Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.
 - 13.5** Add 50 µL of 1X Biotinylated Apolipoprotein E Antibody to each well and incubate for one hour.
 - 13.6** Wash microplate as described above.
 - 13.7** Add 50 µL of 1X SP Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
 - 13.8** Wash microplate as described above.
 - 13.9** Add 50 µL of Chromogen Substrate per well and incubate in ambient light for about 25 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
 - 13.10** Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
 - 13.11** Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be

generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

13.12 Analyze the data as described below.

- 13.12.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- 13.12.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- 13.12.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

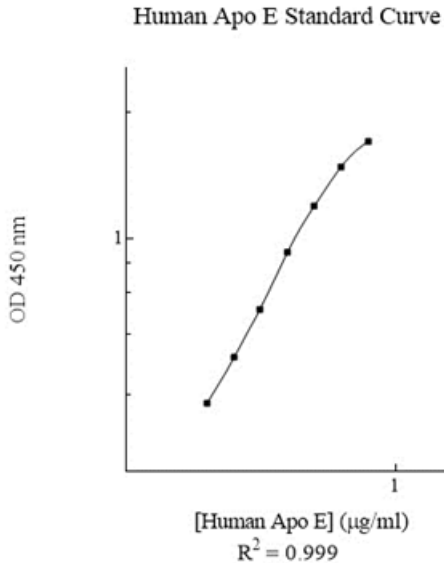


Figure 1. Example of Apolipoprotein E standard curve. The standard curve was prepared as described in Section 10. Background-subtracted data values (mean +/- SD) are graphed.

15. Typical Sample Values

SENSITIVITY –

The minimum detectable dose (MDD) of Apolipoprotein E is typically 3 ng/ml.

PRECISION –

Intra-assay precision was determined by testing replicates of three plasma samples twenty times in one assay.
Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-assay Precision	Inter-Assay Precision
CV (%)	6.4	10.1

RECOVERY –

Standard Added Value	0.016 - 0.125 µg/ml
Recovery (%)	90-109 %
Average Recovery (%)	97 %

LINEARITY OF DILUTION -

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Dilution Factor	Plasma	Serum
1:800	105	90
1:1600	100	101
1:3200	90	110

16. Assay Specificity

This kit recognizes Apolipoprotein E in human plasma, serum and cerebrospinal fluid samples

This kit recognizes Apo E2, Apo E3, and Apo E4 isoforms.

This assay recognizes both natural and recombinant human Apo E.

No significant cross-reactivity observed with Apo A1, Apo A2, Apo A4, Apo A5, Apo B, Apo C1, Apo C2, Apo C3, Apo H, Apo J, and Apo M.

17. Species Reactivity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	None
Equine	None
Mouse	None
Rat	None
Swine	None
Rabbit	None

18.Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4°C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background/ Low sensitivity	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

19. Notes

Technical Support

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