



Human ApoE ELISA Kit

Vertrieb:

L O X O GmbH Immunbiologie Biochemie, Produkte und Systeme
Postfach 11 30 69215 Dossenheim
Telefon +49 (0) 62 21 - 86 80 23 **FAX** +49 (0) 62 21 - 86 80 255
E-Mail: info@loxo.de **Internet:** www.loxo.de

Assaypro LLC
30 Triad South Drive
St. Charles, MO 63304
T (636) 447-9175
F (636) 447-9475
www.assaypro.com

Hinweis/Note:

Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

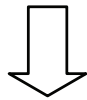
Thank you for choosing Assaypro.

Assay Summary

Add 50 μ l of Standard/ Sample per well.
Incubate 2 hours.



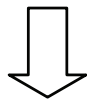
Wash, then add 50 μ l of
Biotinylated Antibody per well.
Incubate 1 hour.



Wash, then add 50 μ l of
SP Conjugate per well.
Incubate 30 minutes.



Wash, then add 50 μ l of
Chromogen Substrate per well.
Incubate 12 minutes.



Add 50 μ l of Stop Solution per well.
Read at 450 nm immediately.

AssayMax Human Apolipoprotein E ELISA Kit

Catalog No. EA8003-1
Sample Insert/Reference Only

Introduction

Apolipoprotein E (ApoE) 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. ApoE is essential for the normal catabolism of triglyceride-rich lipoprotein constituents and cardiovascular diseases (1). ApoE 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. ApoE deficiency causes type III hyperlipoproteinemia and premature atherosclerosis (2, 3). ApoE is a major genetic risk factor for late-onset familial Alzheimer's disease and for cognitive deficits associated with aging (4-7). ApoE-4 enhances HIV-1 cell entry *in vitro* and the ApoE $\epsilon 4/\epsilon 4$ genotype accelerates HIV disease progression (8).

Principle of the Assay

The AssayMax Human Apolipoprotein E ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human ApoE in plasma, serum, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures ApoE in less than 4 hours. A polyclonal antibody specific for human ApoE has been pre-coated onto a 96-well microplate with removable strips. ApoE in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for ApoE, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**

- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human ApoE Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human ApoE.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human ApoE Standard:** Human ApoE in a buffered protein base (1.6 µg, lyophilized, 2 vials).
- **Biotinylated Human ApoE Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against ApoE (140 µl).
- **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard, SP Conjugate, and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 μ l, 20-200 μ l, 200-1000 μ l, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes and assay. Dilute samples 1:400 into EIA Diluent 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).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:400 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Media:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Cell Lysate:** Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml cold PBS with 0.5 M EDTA. Centrifuge suspension at 1500 x *g* for 10 minutes at 4°C and aspirate supernatant. Re-suspend pellet in ice-cold Lysis Buffer (10 mM Tris, pH8.0, 130 mM NaCl, 1% Triton X-100, protease inhibitor cocktail). For every 1×10^6 cells, add approximately 100 μ L of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 x *g* for 30 minutes at 4°C and collect supernatant for assay.
- **CSF:** Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:20 into EIA Diluent and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.

- **Standard Curve:** Reconstitute the 1.6 µg of Human ApoE Standard with 0.8 ml of EIA Diluent to generate a 2 µg/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (2 µg/ml) 1:2 with EIA Diluent to produce 1, 0.5, 0.25, 0.125, 0.0625, and 0.0313 µg/ml solutions. EIA Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C and used within 3 days.

Standard Point	Dilution	[ApoE] (µg/ml)
P1	Standard (2 µg/ml)	2.000
P2	1 part P1 + 1 part EIA Diluent	1.000
P3	1 part P2 + 1 part EIA Diluent	0.500
P4	1 part P3 + 1 part EIA Diluent	0.250
P5	1 part P4 + 1 part EIA Diluent	0.125
P6	1 part P5 + 1 part EIA Diluent	0.063
P7	1 part P6 + 1 part EIA Diluent	0.031
P8	EIA Diluent	0.000

- **Biotinylated Human ApoE Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human ApoE Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with

300 μ l of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.

- Add 50 μ l of Biotinylated Human ApoE Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 μ l of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μ l of Chromogen Substrate per well and incubate for about 12 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.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to yellow.
- 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.

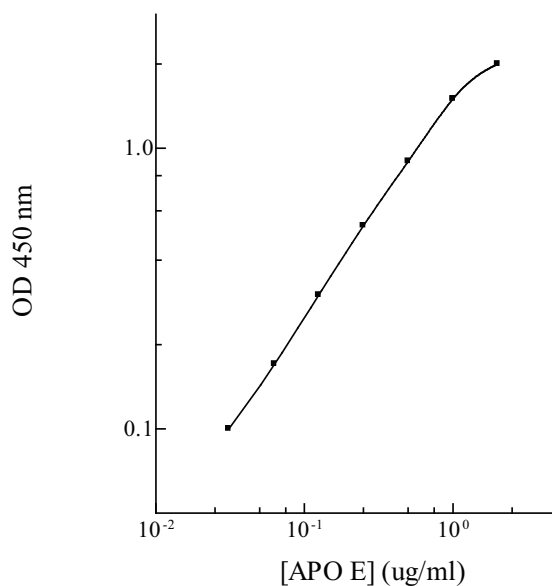
Data Analysis

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

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

Human APO E Standard Curve



Performance Characteristics

- The minimum detectable dose of ApoE is typically ~ 0.03 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.7% and 7.2% respectively.
- The kit recognizes ApoE-2, ApoE-3, and ApoE-4 isoforms.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:200	94%	96%
1:400	98%	99%
1:800	106%	105%

Recovery

Standard Added Value	0.0625 – 0.5 µg/ml
Recovery %	85 – 111%
Average Recovery %	98%

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	<5%
Mouse	None
Rat	None
Rabbit	None
Swine	None
Human	100%

- No significant cross reactivity observed with ApoA-I, ApoA-II, ApoB, ApoC-I, ApoC-II, and ApoC-III.

Reference Value

- On average, normal human ApoE plasma level is 50 µg/ml.

References

- (1) Mahley RW (1988) *Science* 240:622-630
- (2) Lohse P *et al.* (1992) *J. Lipid Res.* 33:1583-1590
- (3) Zhang SH *et al.* (1992) *Science* 258:468-471
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- (7) Schultz MR *et al.* (2008) *Neurology* 70:1771-1777
- (8) Burt TD *et al.* (2008) *Proc. Natl. Acad. Sci. USA* 105:8718-8713

Version 2.6