APOE (Human) ELISA Kit

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96 assays

Version: 32

Intended for research use only

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Introduction

Background

Apolipoprotein E (Apo E) is a 34 kDa polymorphic protein with 299 amino acids. Apo E occurs in all lipoprotein fractions in plasma. It is synthesized primarily by the liver and is a main apoprotein of the chylomicron. Apo E is essential for the normal catabolism of triglyceride-rich lipoprotein constituents and cardiovascular diseases (1). Apo 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: Apo E2, Apo E3, and Apo E4. Apo E3 is normal, whereas Apo E2 and Apo E4 are dysfunctional. Apo E deficiency causes type III hyperlipoproteinemia and premature atherosclerosis (2-3). Apo E is a major genetic risk factor for late-onset familial Alzheimer's disease and for cognitive deficits associated with aging (4-7). Apo E4 enhances HIV-1 cell entry in vitro and the Apo E E4/E4 genotype accelerates HIV disease progression (8).

Principle of the Assay

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

General Information

Materials Supplied

List of component

Component	Amount
Human Apolipoprotein E Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Apo E.	96 (8x12) wells
Sealing Tapes: Pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Human Apolipoprotein E Standard: Human Apo E in a buffered protein base, lyophilized.	1 µg x 2
Biotinylated Human Apolipoprotein E Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human Apo E.	120 µL
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 mL
Standard Diluent (1x): A buffered protein base with stabilizer.	2 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL x 2
SP Conjugate (100x): A 100-fold concentrate.	80 µL
Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine.	7 mL
Stop Solution (1x): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction.	11 mL

Storage Instruction

- ✓ 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), Standard Diluent (1x), 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.

Materials Required but Not Supplied

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

Precautions for Use

- ✓ This product is for Research Use Only and is not intended for use in diagnostic procedures.
- ✓ Prepare all reagents (diluent buffer, wash buffer, standard, 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 insert. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial, the biotinylated antibody vial, and the standard diluent vial before opening and using contents.
- ✓ The Stop Solution is an acidic solution.
- ✓ The kit should not be used beyond the expiration date.

Assay Protocol

Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ MIX Diluent Concentrate (10x): Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, 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. Store for up to 30 days at 2-8°C.
- ✓ Human Apolipoprotein E Standard: Reconstitute the Human Apolipoprotein E Standard (1 µg) with 0.5 mL of Standard Diluent to generate a 2 µg/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (2 µg/mL) 2-fold with equal volume of MIX Diluent to produce 1, 0.5, 0.25, 0.125, 0.063, 0.031, and 0.016 µg/mL solutions. MIX Diluent serves as the zero standard (0 µg/mL). Reconstitute a new vial for each assay.

Standard Point	Dilution	[Apo E] (µg/mL)
P1	1 part Standard (2 µg /mL) + 1 part MIX Diluent	1.0
P2	1 part P1 + 1 part MIX Diluent	0.5
P3	1 part P2 + 1 part MIX Diluent	0.25
P4	1 part P3 + 1 part MIX Diluent	0.125
P5	1 part P4 + 1 part MIX Diluent	0.063
P6	1 part P5 + 1 part MIX Diluent	0.031
P7	1 part P6 + 1 part MIX Diluent	0.016
P8	MIX Diluent	0.0

- ✓ Biotinylated Human Apolipoprotein E Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent to produce a 1x solution. The undiluted antibody should be stored at -20°C.
- ✓ Wash Buffer Concentrate (20x): Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, 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.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Sample Preparation

- ✓ 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 collect plasma. A 400-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. 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 anticoagulant).

- ✓ Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 400-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. A 20-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Cell Culture Supernatant: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into MIX Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

  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 (for reference only; please follow the insert for specific dilution suggested)	
100x	10000x
A) 4 μL sample : 396 μL buffer (100x) = 100-fold dilution Assuming the needed volume is less than or equal to 400 μL.	A) 4 μL sample : 396 μL buffer (100x) B) 4 μL of A : 396 μL buffer (100x) = 10000-fold dilution Assuming the needed volume is less than or equal to 400 μL.
1000x	100000x
A) 4 μL sample : 396 μL buffer (100x) B) 24 μL of A : 216 μL buffer (10x) = 1000-fold dilution Assuming the needed volume is less than or equal to 240 μL.	A) 4 μL sample: 396 μL buffer (100x) B) 4 μL of A : 396 buffer (100x) C) 24 μL of B : 216 μL buffer (10x) = 100000-fold dilution Assuming the needed volume is less than or equal to 240 μL.

Assay Procedure

1. 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).
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.

3. Add 50 µL of Human Apolipoprotein E standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µL of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µL of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µL of Biotinylated Human Apolipoprotein E Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 20 minutes or until the optimal blue color density develops.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thoroughly mixing. Break any bubbles that may have formed.
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.

✓ Assay Summary

1. Add 50 µL of Standard or Samples per well. Incubate 2 hours.
2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour.
3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.
4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 20 minutes.
5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

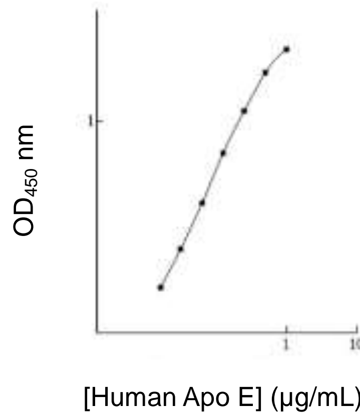
- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression 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.
- ✓ Typical Data

  The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be cause by technique differences.

Standard Point	μg/mL	OD	Average OD
P1	1.0	2.395 2.525	2.460
P2	0.5	1.879 1.797	1.838
P3	0.25	1.103 1.167	1.135
P4	0.125	0.689 0.645	0.667
P5	0.063	0.335 0.383	0.359
P6	0.031	0.195 0.207	0.201
P7	0.016	0.120 0.128	0.124
P8	0.0	0.037 0.029	0.033
Sample: Pooled Normal Sodium Citrate Plasma (400x)		0.774 0.714	0.744

- ✓ Standard Curve

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



- Reference Value

  Plasma and serum samples from healthy adults were tested (n=40). On average, human Apo E level was 58.8 μg/mL.

Performance Characteristics

- The kit recognizes Apo E2, Apo E3, and Apo E4 isoforms.
- This assay recognizes both natural and recombinant human Apo E.
- The minimum detectable dose of human Apo E as calculated by 2SD from the mean of a zero standard was established to be 4.3 ng/mL.
- Intra-assay precision was determined by testing 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		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	5.7%	5.1%	6.3%	10.2%	9.6%	10.1%
Average CV (%)	5.7%			10.0%		

- Recovery

Standard Added Value	0.063-0.5 μg/mL
Recovery %	90-109%
Average Recovery %	97%

- Linearity

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

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
200x	95%	96%
400x	99%	101%
800x	107%	106%

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✓ Cross-Reactivity

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

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.

10% FBS in culture media will not affect the assay.

Resources

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.
	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.

Deficient Standard Curve Fit	Non-optimal sample dilution	Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples.
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

References

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Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												