INTENDED USE

The **Human Apolipoprotein E (ApoE) ELISA Kit** is a sandwich immunoassay for the quantitative measurement of human ApoE in serum, plasma, cell culture supernatants, cerebrospinal fluid (CSF), and other validated sample matrices.

This kit is intended for research use only (RUO) and not for diagnostic or therapeutic applications.

GENERAL INFORMATION

Apolipoprotein E (ApoE) is a lipid-binding protein involved in the transport and metabolism of cholesterol and other lipids. It is primarily produced by the liver and macrophages in peripheral tissues, while astrocytes are the main source of ApoE in the central nervous system, where it serves as the principal cholesterol carrier in the brain.

Human ApoE has three major isoforms: ApoE2, ApoE3, and ApoE4. ApoE4 is a well-established genetic risk factor for late-onset Alzheimer's disease and may influence the accumulation and clearance of amyloid-beta 42 (Aβ42), a peptide associated with the formation of amyloid plaques. ApoE is also involved in lipid metabolism and is relevant to research on cardiovascular and neurodegenerative diseases.

ApoE interacts with lipoprotein receptors to facilitate the uptake and distribution of lipids among cells. In the central nervous system, this process is essential for maintaining neuronal membranes, synaptic function, and cellular repair. ApoE expression and concentration can vary depending on tissue type, physiological conditions, and disease state, making it an important target for investigating lipid homeostasis and its relationship to human health.

PRINCIPLE OF THE TEST

The Human ApoE ELISA kit is based on the binding of human ApoE in samples to two antibodies, one immobilized on the microwells, and the other conjugated to horseradish peroxidase (HRP). After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of human ApoE present in the sample.

Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The concentration of human ApoE in samples is calculated from a standard curve of standards prepared from purified ApoE.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Apolipoprotein E Standard Cat. No. APOE-092, 1.2 ml	One vial containing ApoE in buffer with proteins and antimicrobial. Refrigerate vial, or store frozen for long term stability.
Prepare 2-fold dilutions using **Working Sample Diluent**, as follows; sufficient for two entire curves:	
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Human ApoE HRP Conjugate Concentrate (100x) Cat. No. H-APOE-093, 0.15ml	In buffer with protein, detergents, and antimicrobial. Dilute fresh as needed; 10 ul of concentrate to 1 ml of Working Sample Diluent (WSD) is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Standard	+ Diluent =	Final Concn
200 ng/ml Standard	None	200 ng/ml
220 ul of 200 ng/ml	220 ul	100 ng/ml
220 ul of 100 ng/ml	220 ul	50 ng/ml
220 ul of 50 ng/ml	220 ul	25 ng/ml
220 ul of 25 ng/ml	220 ul	12.5 ng/ml
220 ul of 12.5 ng/ml	220 ul	6.25 ng/ml
220 ul of 6.25 ng/ml	220 ul	3.125 ng/ml
Use the same day of testing, and/or store frozen.		

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Anti-ApoE Coated Strip Plate	APOE-091	8-well strips (12)	Coated with Anti-ApoE and post-coated with stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Culture medium, serum, and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For all samples, clarify by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, store frozen for long-term storage.

Assay Validation

Validate the performance of the ApoE sample and matrix in the assay system for recovery (see Limits of the Assay, page 6), as follows:

Recovery – a measure of the interference of the sample matrix in providing accurate quantitation of ApoE in the sample relative to the ApoE Standards.

Prepare and run a series of dilutions of the ApoE sample (concentrations that will fall within the Standard range) in Working Sample Diluent to determine the dilutions that give consistent and accurate quantitation. Non-human serum and plasma may require a 1:20 dilution or greater to obtain consistent quantitation or complete recovery. Full Recovery from processing solutions will likely require much less dilution. Serum and plasma require at least a 1:500 dilution to obtain consistent quantitation or complete recovery. Samples reading above the top standard should be diluted further and re-tested.

Plate Set-up

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 14 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes.
- Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of working sample diluent (blank), standards, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for **60 minutes.**
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 60 min; 5 washes]

- Add 100ul of diluted **Anti-Human ApoE HRP Conjugate** to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes, or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

CALCULATION OF RESULTS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, ApoE concentrations may be determined as follows:

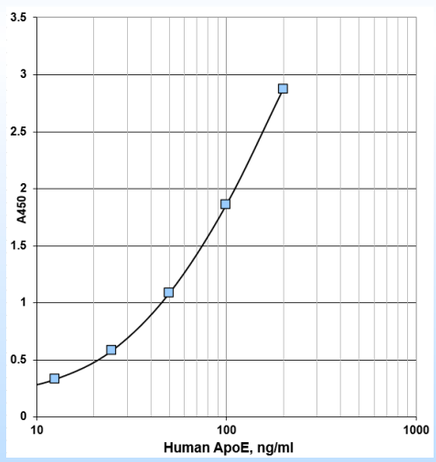
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of ApoE (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The ApoE concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor.
5. Samples producing signals higher than the 200 ng/ml standard should be further diluted and re-assayed.

Typical Results:

Wells	Calibrators	A450 nm
A 1,2	**Negative Diluent Blank**	0.07
B 1,2	3.125 ng/ml **Standard**	0.14
C 1,2	6.25 ng/ml **Standard**	0.22
D 1,2	12.5 ng/ml **Standard**	0.33
E 1,2	25 ng/ml **Standard**	0.58
F 1,2	50 ng/ml **Standard**	1.08
G 1,2	100 ng/ml **Standard**	1.86
H 1,2	200 ng/ml **Standard**	2.87
A 3,4	**Sample** [Diluted 1:1,000]	1.68

Sample result: 85 ng

Calculated: 1,000 fold dilution x 85ng = **85** ug/ml



PERFORMANCE CHARACTERISTICS

Specificity

This ELISA antibody pair detects human ApoE (isoforms ApoE2, ApoE3 and ApoE4).

Sensitivity

The minimum detectable dose of Human Apo E is **~1 ng/mL**. This was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

Precision

To determine inter & intra-assay precision, 2 samples for each standard curve are run at a high and low point on the standard curve. The standard curve concentration points as well as the samples are tested on a single plate. Two different concentration values are obtained for each sample. The two concentration values for each sample is compared to each other using the CV% calculation. **Intra & Inter-Assay CV: <10%**

Sample values

Serum/Plasma - Ten serum samples from apparently healthy status were evaluated for the presence of Human ApoE in this assay. All samples measured **above 10 ug/ml**. No medical histories were available for the donors used in this study. Samples typically range in value from **20-80 ug/ml**. A starting dilution of **1:500-1:1000** is recommended to bring the majority of samples within range of the standard curve.

Standards

The immunoassay is calibrated using a highly purified ApoE protein purified through a multi-step precipitation process natively from Human serum.

LIMITS OF THE ASSAY

1) For Research Use only. Not for use in Diagnostic procedures.

2) Do not use the kit beyond the expiration date on the label.

3) Do not mix or use reagents from other lots or sources.

4) If a sample generates a value higher than the 200 ng/ml standard, further dilute the sample to bring it within range of the assay.

5) Variations in sample collection, processing, and storage may cause sample value differences.

6) The possibility of interference of other factors in biological samples cannot be excluded.

QUALITY CONTROL

Reagents: Accurate and reproducible assay results rely on proper storage, handling, and control of reagent and sample temperature. Store all reagents as indicated and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Standard Curve: The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-uniform or low signals may indicate problems with technique, protocol directions, and/or reagent preparation, use, or stability. Do not rely on results generated from an assay with these issues.

Technique: Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing, and handling of samples and reagents.

Equipment: Precision of results relies on uniform and effective washing techniques; an automatic washer may be used. ELISA reader and pipettes should be properly calibrated.

Similar materials available from ADI

Catalog#	Description
APOE11-C	Recombinant Human ApoE protein W. Blot +ve control
APOE11-S	Goat Anti-Human ApoE protein antiserum #1
APOE12-C	Recombinant Human ApoE protein W. Blot +ve control
APOE13-A	Rabbit Anti-Human Plasma Apolipoprotein E (ApoE) IgG, aff pure
APOE13-C	Human plasma Apolipoprotein E protein control for WB
APOE15-R	Human Purified native plasma Apolipoprotein E protein
APOE25-R	Recombinant (E. coli) Purified Human Apolipoprotein E2 protein
APOE31-C	Human ApoE3 protein W. Blot +ve control
APOE31-S	Rabbit Anti-Human ApoE3 protein antiserum #1
APOE35-R	Recombinant Purified Human Apolipoprotein E3 protein
APOE36-R	Recombinant (E coli) Purified Human Apolipoprotein E3 protein
APOE45-R	Recombinant Purified Human Apolipoprotein E4 protein
APOJ11-A	Goat Anti-Human ApoJ (Clusterin/Apolipoprotein J/Apo J) protein IgG aff pure
APOJ11-C	Recombinant purified Human ApoJ (Clusterin/Apolipoprotein J) protein control for WB
APOJ13-C	Human Plasma Apolipoprotein J protein control for WB
APOJ15-N-10	Apolipoprotein J, Human Plasma
APOJ35-R-10	Recombinant (E.Coli) purified Rat ApoJ (Clusterin/Apolipoprotein J) protein for ELISA
APOJ36-R-10	Recombinant (HEK 293) purified Human ApoJ (Clusterin/Apolipoprotein J) protein for ELISA

Instruction Manual No. M-APOE-096

Human Apolipoprotein E (ApoE) ELISA Kit

Cat. #APOE-096, 96 Tests

For Quantitation of Human Apolipoprotein in Serum, Plasma, and other Biological Solutions

For research use only (RUO), not for diagnosis, cure, or prevention of the disease.

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 W • San Antonio • Texas 78249 • USA
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: service@4adi.com
www.4adi.com

ELISA Kit Components	Amount	Part
Anti-ApoE Coated Microwell Plate	8-well strips (12)	APOE-091
Apolipoprotein E Standard	1.2 ml	APOE-092
Anti- Human ApoE HRP Conjugate (100X)	0.15 ml	H-APOE-093
Sample Diluent Concentrate (20x)	10 ml	SD20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-APOE-096