Human APOE Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE50006

Size: 96T

Sensitivity: 0.03 ng/mL

Range: 0.156-10 ng/mL, 0.313-20 ng/mL

Usage: For the quantitative detection of human APOE concentrations in serum, plasma, cell culture supernatant, urine and human milk.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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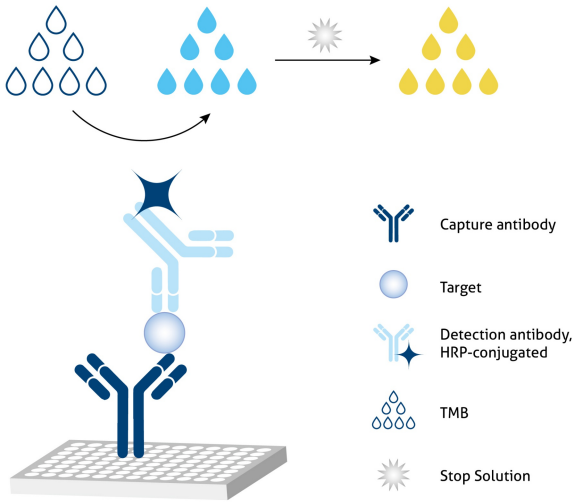
1. Background

APOE (apolipoprotein E) is a plasma protein implicated in lipid metabolism. It is produced predominantly by hepatocytes, macrophages, and neural cells. In the CNS, APOE is the major extracellular lipid carrier and plays a key role in neuronal protection and repair after injury. APOE is polymorphic, with three major alleles: APOE2 (cys112, cys158), APOE3 (cys112, arg158), and APOE4 (arg112, arg158). APOE2 plays a protective role against both Alzheimer's (AD) and heart disease. APOE4 confers a higher risk for atherosclerosis and Alzheimer's disease. Analysis of cerebrospinal fluid (CSF) and plasma APOE protein levels in AD patients had been performed to examine its association with AD pathology. In old age, high plasma APOE levels precede an increase of circulating CRP and strongly associates with cardiovascular mortality.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with HRP)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

3.8 Horizontal orbital microplate thermostatic shaker.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 40 ng/bottle; lyophilized	2 bottles	
Detection antibody, HRP-conjugated (100×) - 60 µL/vial*	1 vial	
Sample Diluent PT 1 - 30 mL/bottle. For human serum, plasma and cell culture supernatant.	2 bottle	
Sample Diluent PT 4B1 - 30 mL/bottle. For urine and human milk.	1 bottle	
Detection Diluent - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.4 Urine: Collect urine samples and centrifuge for 20 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.5 Human Milk: Collect milk samples and Centrifuge for 15 minutes at 1000xg at 2-8°C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

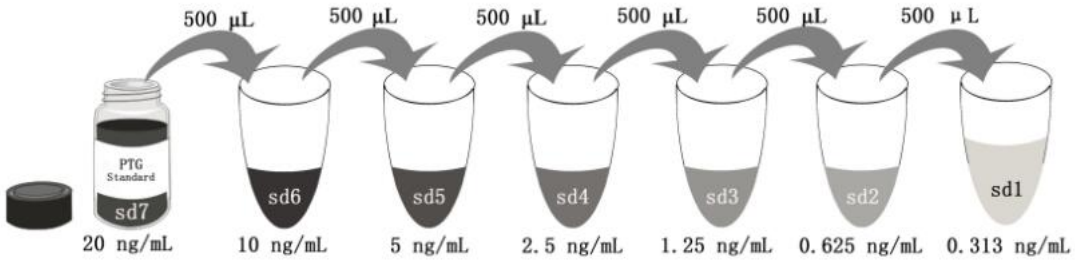
7.2 Detection Antibody, HRP-conjugated(1X): Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody, HRP-conjugated + 990 µL Detection Diluent (Centrifuge the 100X Detection Antibody solution, HRP-conjugated for a few seconds prior to use)

7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:16,000 or 1:32,000 is recommended for human serum and plasma; 1:80 or 1:160 is recommended for cell culture supernatant; 1:2 or 1:4 is recommended for urine; 1:80 or 1:160 is recommended for human milk.

7.4 Standard Serial Dilution:

For human serum, plasma and cell culture supernatant add 2 mL Sample Diluent PT 1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

For urine and human milk add 4 mL Sample Diluent PT 4B1 in protein standard.

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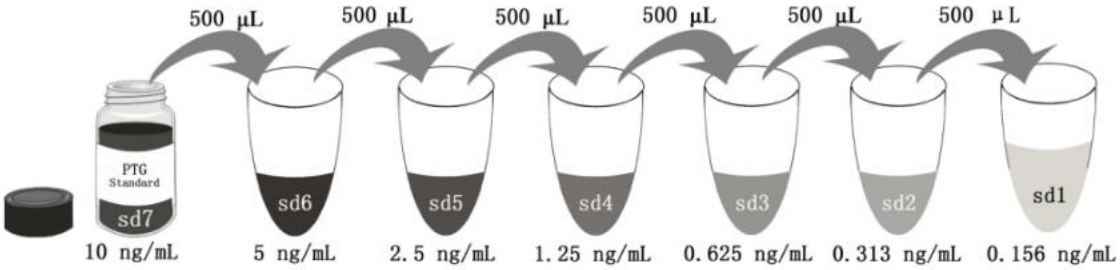

Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4B1	**4000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated can be used immediately). 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.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 50 µL of each standard and sample to the appropriate wells. At the same time, add 50 µL of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation7.2) to each well. (Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate on a microplate thermostatic shaker for 1 hour at 37°C, 500±50 rpm.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.6 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.7 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples Diluent Antibody Solution	50 µL each	60 min	4 times	Cover Wells incubate at 37°C, 500±50 rpm
2	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
3	Stop Solution	100 µL	0 min	Do not wash	-
4	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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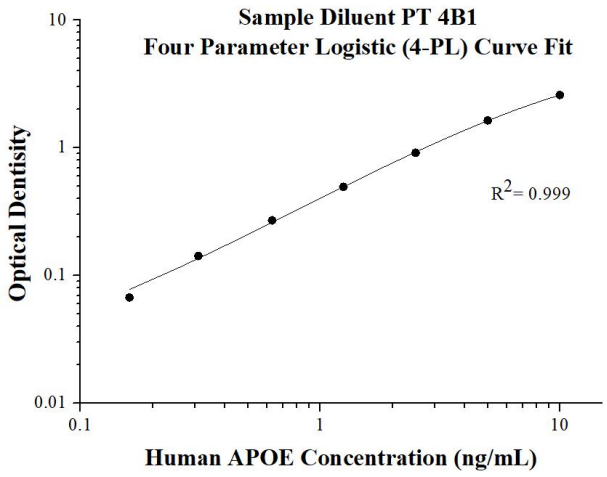
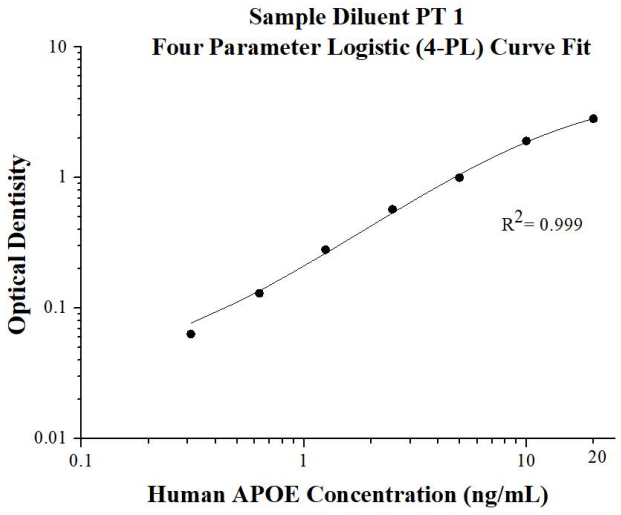
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.0125 0.013	0.0128	0
0.156	0.0847 0.0757	0.0802	0.0675
0.313	0.1546 0.1561	0.1554	0.1426
0.625	0.2827 0.2846	0.2837	0.2709
1.25	0.5223 0.4962	0.5093	0.4965
2.5	0.9306 0.9266	0.9286	0.9159
5	1.6834 1.6255	1.6545	1.6417
10	2.6561 2.5616	2.6089	2.5961



(ng/mL)	O.D	Average	Corrected
0	0.0122 0.0113	0.0118	0
0.313	0.0691 0.0812	0.0752	0.0634
0.625	0.1424 0.1412	0.1418	0.1301
1.25	0.2978 0.2878	0.2829	0.2811
2.5	0.5991 0.5693	0.5842	0.5725
5	0.9638 1.0615	1.0127	1.0009
10	1.9757 1.8658	1.9208	1.9090
20	2.9354 2.7596	2.8475	2.8358

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	20	9.66	0.18	1.88
2	20	1.73	0.04	2.38
3	20	0.41	0.02	3.97

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	9.38	0.19	2.08
2	24	1.76	0.04	2.15
3	24	0.46	0.01	2.15

9.3 Recovery

The recovery of human APOE spiked to three different levels throughout the range of the assay in various matrice were evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:64,000	103	82-119
	1:12,8000	102	82-126
Cell culture supernatant	1:160	106	90-115
	1:320	104	81-126
Urine	1:8	99	80-113
	1:16	95	81-110
Human milk	1:320	108	97-121
	1:640	100	89-121

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9.4 Sample values

Human serum - Human serum samples were evaluated for the presence of human APOE in this assay.

Sample Type	Mean (**ug/mL**)	Range (**ug/mL**)
Human serum (n=16)	72.68	17.06-118.38

Cell culture supernatant -HepG2 (human hepatocellularcarcinoma cells) were cultured in DMEM supplemented with 10% fetal bovine serum, 2.5 mM L- glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human APOE, and measured 41.30 ng/mL.

Urine/Human milk - Urine and human milk samples were evaluated for the presence of human APOE in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Urine (n=7)	6.56	2.35-20.75
Human milk (n=7)	76.11	44.74-137.34

9.5 Sensitivity

The minimum detectable dose of human APOE is 0.03 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The human serum samples were initially diluted 1:8,000; cell culture supernatant was initially diluted 1:20; human milk was initially diluted 1:40.)

		Human serum (Sample Diluent PT 1)	Cell culture supernatant (Sample Diluent PT 1)	Urine (Sample Diluent PT 4B1)	Human milk (Sample Diluent PT 4B1)
1:2	Average% of Expected	100	100	100	100
	Range (%)	-	-	-	-
1:4	Average% of Expected	92	92	113	103
	Range (%)	86-99	83-100	106-121	95-115
1:8	Average% of Expected	93	88	117	103
	Range (%)	83-99	82-95	106-124	96-116
1:16	Average% of Expected	101	88	105	93
	Range (%)	85-119	79-100	83-116	91-97

10. References

1. Teng E. et al. (2015). Dement Geriatr Cogn Disord. 39(3-4):154-66.

2. Mooijaart SP. et al. (2006). PLoS Med. Jun;3(6):e176.

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