

Human APOE Sandwich ELISA Kit Datasheet

For the quantitative detection of human APOE concentrations in serum, plasma, urine, saliva and human cerebrospinal fluid.

General Information

Catalogue Number	KE00156
Product Name	Human APOE Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	78.1-5000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	348
SwissProt	P02649

Kit Components & 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 - 10000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4 - 30 mL/bottle. For serum, plasma, urine and human cerebrospinal fluid samples	2 bottles	
Sample Diluent PT 1 - 30 mL/bottle. For saliva sample	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

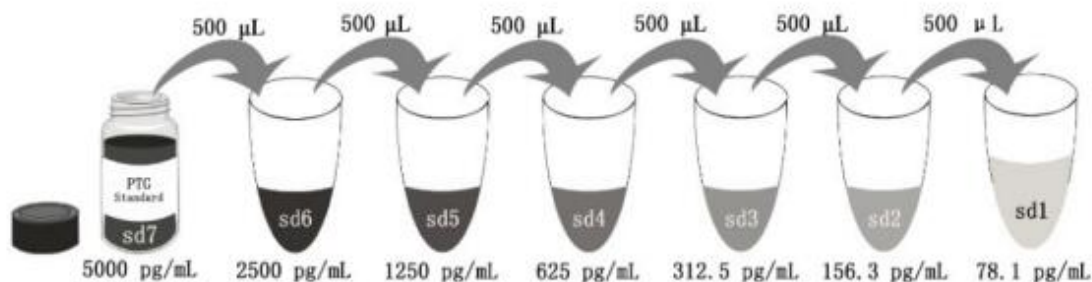
NB: Do not use the kit after the expiration date.

Sample Diluent PT 4 is for protein standard, serum, plasma, urine and human cerebrospinal fluid samples.

Sample Diluent PT 1 is for protein standard and saliva sample.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 2 mL Sample Diluent PT 4 or PT 1 in protein standard. This reconstitution gives a stock solution of 5000 pg/mL.



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 PT4 or PT 1	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00156 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The APOE ELISA kit is to be used to detect and quantify protein levels of endogenous APOE. The assay recognizes human APOE. An antibody specific for APOE has been pre-coated onto the microwells. The APOE protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for APOE is added to detect the captured APOE protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

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/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.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:16,000 or 1:32,000 dilution is recommended for

serum or plasma, 1:4 or 1:8 dilution is recommended for urine, 1:4 or 1:8 dilution is recommended for saliva, 1:800 or 1:1,600 dilution is recommended for human cerebrospinal fluid.

Safety Notes

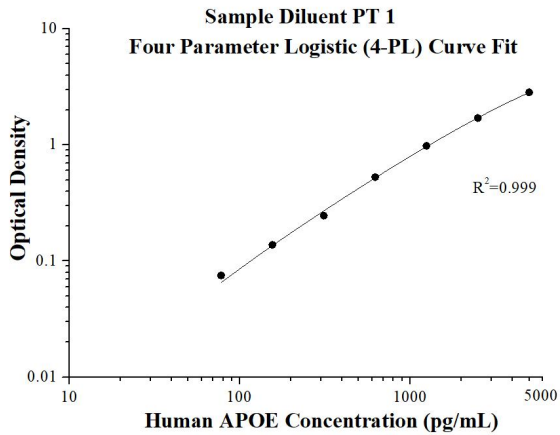
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

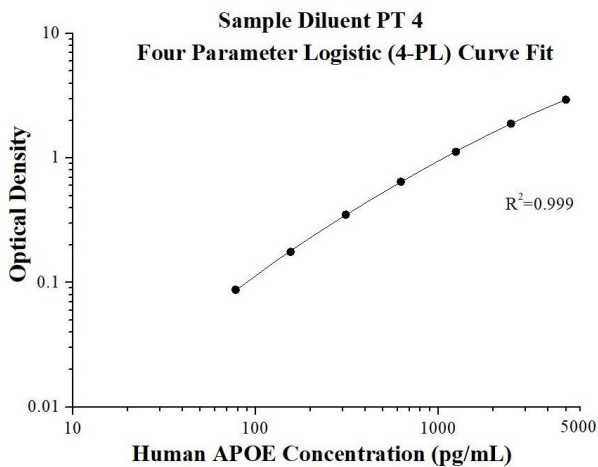
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

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



(pg/mL)	O.D	Average	Corrected
0	0.045 0.047	0.046	-
78.1	0.123 0.119	0.121	0.075
156.3	0.183 0.184	0.183	0.137
312.5	0.294 0.289	0.291	0.245
625	0.590 0.558	0.574	0.528
1250	1.019 1.033	1.026	0.980
2500	1.722 1.777	1.749	1.703
5000	2.852 2.905	2.878	2.832



(pg/mL)	O.D	Average	Corrected
0	0.03 0.029	0.029	-
78.1	0.102 0.132	0.117	0.087
156.3	0.191 0.22	0.205	0.176
312.5	0.358 0.402	0.38	0.350
625	0.641 0.706	0.673	0.644
1250	1.166 1.143	1.154	1.125
2500	1.923 1.915	1.919	1.889
5000	2.99 2.954	2.972	2.942

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 (pg/mL)	SD	CV%
1	20	2,811.2	88.6	3.2
2	20	776.0	23.9	3.1
3	20	291.0	7.4	2.5

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	2,846.8	200.8	7.1
2	24	729.6	22.3	3.1
3	24	283.9	11.1	3.9

Recovery

The recovery of APOE spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:32,000	105	87-118
	1:64,000	95	81-116
Saliva	1:8	99	77-125
	1:16	111	97-125
Urine	1:8	116	109-123
	1:16	111	99-118
Human cerebrospinal fluid	1:3,200	76	73-79
	1:6,400	85	75-96

Sample Values

Samples from healthy volunteers were evaluated for APOE in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human serum (n=16)	26,155	8,013-43,244
Human plasma (n=16)	22,498	9,489-65,868
Saliva (n=8)	3.59	1.15-7.03
Urine (n=8)	2.19	0.47-4.54
Human cerebrospinal fluid (n=1)	2,508	-

Sensitivity

The minimum detectable dose of human APOE is 29.4 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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 serum were initially diluted 1:4,000. Human cerebrospinal fluid were initially diluted 1:200)

		Human serum (PT 4)	Saliva (PT 1)	Urine (PT 4)	Human cerebrospinal fluid (PT 4)
1:2	Average% of Expected	100	-	-	100
	Range (%)	-	-	-	-
1:4	Average% of Expected	94-96	100	100	85-129
	Range (%)	96	-	-	99
1:8	Average% of Expected	79-95	94-118	108	105
	Range (%)	88	107	102-113	93-126
1:16	Average% of Expected	90-98	96-116	116	95
	Range (%)	93	109	108-123	86-102

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.