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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human APOD in serum, plasma.

general information

Catalogue Number	KE00067
Product Name	APOD ELISA Kit
Species cross-reactivity	Human APOD
Range (calibration Range)	0.313 - 20 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**347** (Human)
SwissProt	**P05090** (Human)

kit components & storage

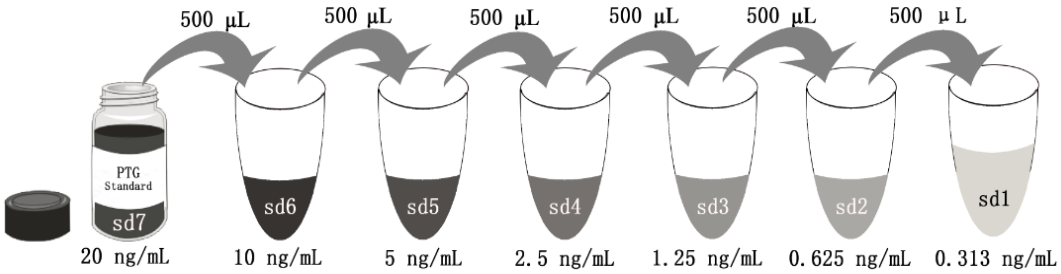
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 40 ng/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ac - 30 mL/bottle; For serum, plasma samples	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

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

Sample Diluent PT 1-ac is for Standard and serum, plasma samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

*Add 2 mL Sample Diluent PT 1-ac in Standard, This reconstitution gives a stock solution of 20 ng/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 PT 1-ac	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00067 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The APOD ELISA kit is to be used to detect and quantify protein levels of endogenous APOD. The assay recognizes human APOD. A polyclonal antibody specific for APOD has been pre-coated onto the microwells. The APOD protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for APOD is added to detect the captured APOD protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody 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 450nm.

background

Apolipoprotein D (ApoD) is a member of the lipocalin superfamily of ligand transporters, and has been implicated in the transport of small hydrophobic molecules. ApoD is also a component of plasma high-density lipoproteins (HDL). Alteration of ApoD expression has been linked to multiple neurological disorders, including Alzheimer's disease. This kit is used to quantify the ApoD level.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

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.

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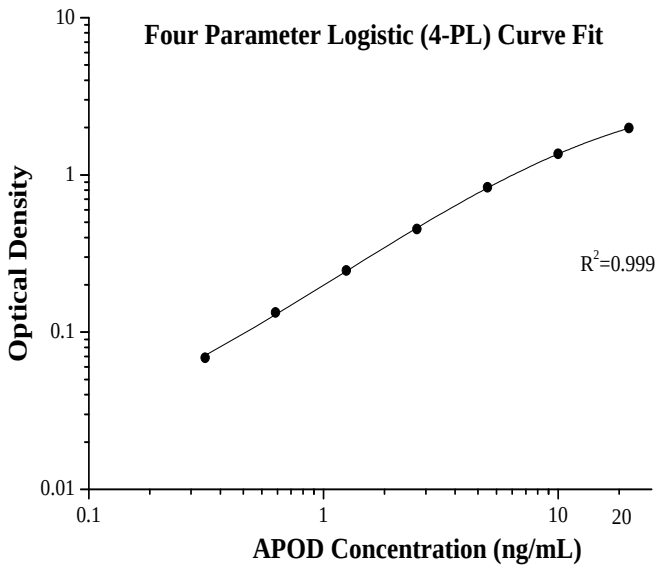
assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 min	4 times	Cover Wells
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells
4	TMB Substrate	100 µL	15-30 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.				

typical data

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	0.013	0.014	—
	0.015		
0.313	0.083	0.0825	0.0685
	0.082		
0.625	0.151	0.147	0.133
	0.143		
1.25	0.273	0.2605	0.2465
	0.248		
2.5	0.463	0.4665	0.4525
	0.47		
5	0.824	0.847	0.833
	0.87		
10	1.353	1.3725	1.3585
	1.392		
20	2.008	1.998	1.984
	1.988		

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			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ng/ml)	11.3	3.1	0.8	11.8	3.4	0.8
SD	0.7	0.2	0.07	0.9	0.2	0.03
CV%	6.4	6.5	9	7.8	6.2	4.7

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recovery

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

Sample Type		Average % of Expected	Range(%)
Citrate plasma	1:4	86	76-96
	1:8	97	93-102

sensitivity

The minimum detectable dose of human APOD is 0.01 ng/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, three samples were spiked with high concentrations of APOD in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:3)

		Citrate plasma
1:2	Average% of Expected	85
	Range(%)	82-89
1:4	Average% of Expected	88
	Range(%)	83-92
1:8	Average% of Expected	98
	Range(%)	97-98
1:16	Average% of Expected	99
	Range(%)	93-105

references

1. Dassati S, et al. Apolipoprotein D takes center stage in the stress response of the aging and degenerative brain. Neurobiol Aging. 2014 Jul;35(7):1632-42.
2. Navarro A, et al. Lifelong expression of apolipoprotein D in the human brainstem: correlation with reduced age-related neurodegeneration. PLoS One. 2013 Oct 22;8(10):e77852.