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Antibodies | ELISA kits | Proteins

Human APOL1 sandwich ELISA kit datasheet

For the quantitative detection of human APOL1 in serum, plasma and urine.

general information

Catalogue Number	KE00047
Product Name	Human APOL1 ELISA Kit
Species cross-reactivity	Human APOL1
Range (calibration Range)	0.156 - 10 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**8542** (Human)
SwissProt	**Q2KHQ6** (Human)

kit components & storage

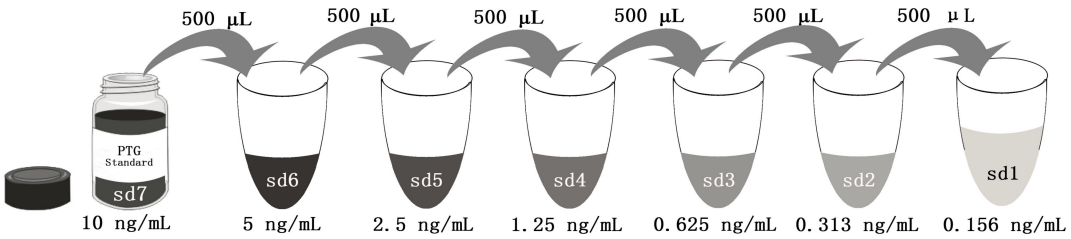
Microplate - antibody coated 96 - well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Protein standard - 20 ng/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1 - 30 mL/bottle	2 bottles	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 is for protein standard and samples.

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

*Add **2 mL** Sample Diluent PT 1 in protein standard. This reconstitution gives a stock solution of 10 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	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00047 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The APOL1 ELISA kit is to be used to detect and quantify protein levels of endogenous APOL1. The assay recognizes human APOL1. An antibody specific for APOL1 has been pre-coated onto the microwells. The APOL1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for APOL1 is added to detect the captured APOL1 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 450 nm with the correction wavelength set at 630 nm.

background

Human apolipo-protein L1 (APOL1) is a minor component of plasma high density lipoprotein (HDL) particles. The human ApoL protein family was thought to be predominantly involved in lipid transport and metabolism. APOL1 is also involved in host innate immunity against Trypanosoma parasites. Once activated, APOL1 can lyse the parasite and protect human from infection. Genetic variants in APOL1 gene, which are found in African ancestry with high frequency, associate with chronic kidney disease, like focal segmental glomerulosclerosis (FSGS), HIV-associated nephropathy (HIVAN), and hypertensive nephropathy. This kit is used to quantify APOL1 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 1:10,000 is suggested according to the individual samples. The urine samples could be test directly with no dilution or 2 times dilution.

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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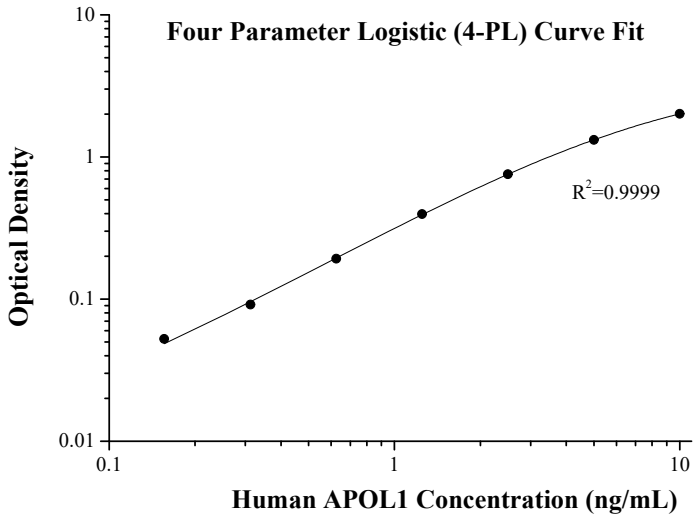
assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	**60 min**	4 times	Cover Wells 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.				

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	0.019	0.0195	—
	0.02		
0.156	0.079	0.072	0.0525
	0.065		
0.313	0.105	0.111	0.0915
	0.117		
0.625	0.212	0.2115	0.192
	0.211		
1.25	0.419	0.416	0.3965
	0.413		
2.5	0.757	0.774	0.7545
	0.791		
5	1.326	1.3385	1.319
	1.351		
10	2.043	2.03	2.0105
	2.017		

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)	8.31	2.21	0.63	9.12	2.31	0.62
SD	0.312	0.095	0.013	0.387	0.073	0.023
CV%	3.8	4.3	2.1	4.2	3.2	3.6

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recovery

The recovery of APOL1 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated. (The plasma samples were initially diluted 1:50,000)

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	94	87-107
	1:4	91	85-101
Urine	1:2	104	83-127
	1:4	106	93-116

sample value

Serum Samples from healthy volunteers were evaluated for APOL1 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)
serum (n=24)	61,000	22,000-133,000
urine (n=6)	7	2-19

sensitivity

The minimum detectable dose of human APOL1 is 0.07 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 containing concentrations of APOL1 in various matrices and diluted with the appropriate **Sample Diluent PT 1** to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:10,000)

		Human plasma	Urine
1:1	Average% of Expected	-	-
	Range (%)	-	-
1:2	Average% of Expected	85	108
	Range (%)	80-89	98-117
1:4	Average% of Expected	97	102
	Range (%)	93-101	87-116
1:8	Average% of Expected	98	101
	Range (%)	92-102	86-122
1:16	Average% of Expected	96	-
	Range (%)	94-108	-

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references

1. Thomson, R., et al. Hydrodynamic gene delivery of baboon trypanosome lytic factor eliminates both animal and human-infective African trypanosomes. Proc Natl Acad Sci U S A. 106: 19509-14 (2009).
2. Friedman, D., *et al*. Genetics of kidney failure and the evolving story of APOL1. J Clin Invest. 121: 3367-74 (2011).

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