Human APOB Sandwich ELISA Kit Datasheet

For the quantitative detection of human APOB concentrations in serum, plasma, cell culture supernatants and human milk.

General Information

Catalogue Number	KE00158
Product Name	Human APOB Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	3.9-250 ng/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	338
SwissProt	P04114

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 - 250 ng/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 1-ec - 30 mL/bottle. For serum, plasma and cell culture supernatants samples	2 bottles	
Sample Diluent PT 3 - 30 mL/bottle. For human milk 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	

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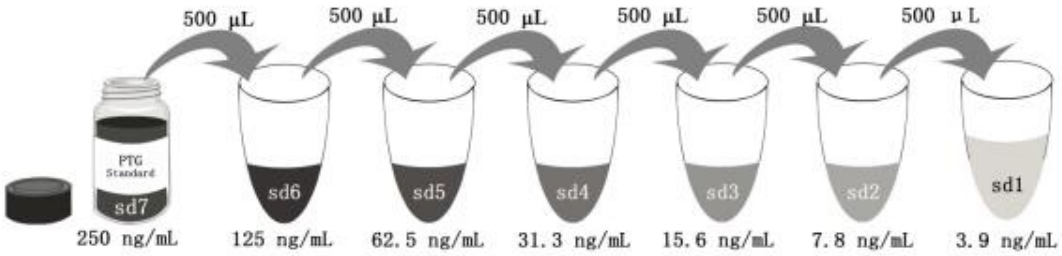
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ec is for protein standard, serum, plasma and cell culture supernatants.
Sample Diluent PT 3 is for protein standard and human milk.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 1-ec or PT 3 in protein standard. This reconstitution gives a stock solution of 250 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-ec or PT 3	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00158 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The APOB ELISA kit is to be used to detect and quantify protein levels of endogenous APOB. The assay recognizes human APOB. An antibody specific for APOB has been pre-coated onto the microwells. The APOB protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human APOB is added to detect the captured human APOB 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

The apolipoprotein B (APOB) is a plasma protein synthesized primarily in the liver and intestine and play an important role in lipid and cholesterol metabolism. The APOB gene encodes two different isoproteins through mRNA editing, APOB-48 and APOB-100. APOB-100 is synthesized exclusively by the liver and is essential for the assembly of VLDL in the liver. APOB-48 is synthesized exclusively by the small intestine and is essential for the assembly of chylomicrons in the intestine. APOB-48 and APOB-100 share a common N-terminal sequence, but APOB-48 lacks APOB-100's C-terminal LDL receptor binding region. Plasma APOB levels are associated with coronary heart disease.

Sample Preparation

Different samples may require proper dilution to fall within the range of the assay. The serum or plasma is better to be diluted 1:20,000 or 1:40,000 before assay (The serum or plasma samples should be treated with Triton-X 100. Dilute samples 2x with 1% Triton-X 100 followed by vortex for 5 seconds), 1:2 or 1:4 dilution is recommended for cell culture supernatants, 1:50 or

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1:100 dilution is recommended for human milk.

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.				

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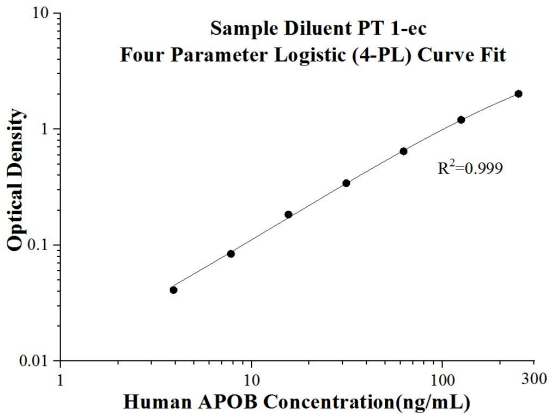
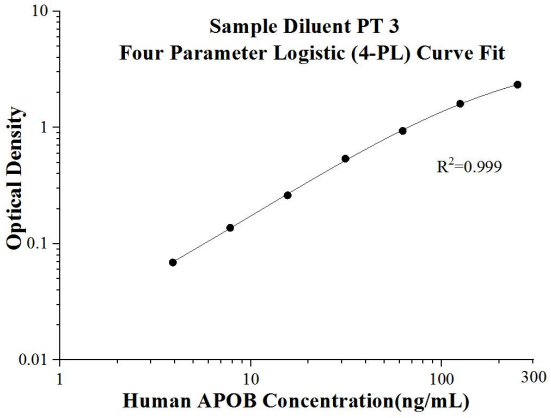
Example data

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	0.088 0.082	0.085	-
3.9	0.131 0.121	0.126	0.041
7.8	0.170 0.167	0.169	0.084
15.6	0.275 0.262	0.269	0.184
31.3	0.420 0.434	0.427	0.342
62.5	0.727 0.732	0.73	0.645
125	1.303 1.276	1.29	1.205
250	2.092 2.113	2.103	2.018



(ng/mL)	O.D	Average	Corrected
0	0.108 0.108	0.108	-
3.9	0.178 0.175	0.177	0.069
7.8	0.247 0.243	0.245	0.137
15.6	0.375 0.363	0.369	0.261
31.3	0.651 0.643	0.647	0.539
62.5	1.026 1.058	1.042	0.934
125	1.701 1.711	1.706	1.598
250	2.450 2.439	2.445	2.337

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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	119.9	4.1	3.4
2	20	28.3	0.7	2.6
3	20	7.2	0.4	5.4

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	133.3	9.8	7.3
2	24	31.0	2.4	7.9
3	24	8.0	0.8	10.0

Recovery

The recovery of APOB 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	1:80,000	94	80-109
	1:160,000	96	77-121
Cell culture supernatants	1:2	104	94-114
	1:4	102	86-122
Human milk	1:200	103	81-120
	1:400	100	75-122

Sample Values

Serum and human milk samples from healthy volunteers were evaluated for APOB in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean **(ug/mL)**	Range **(ug/mL)**
Human serum (n=25)	576.2	147.4-1,966.7
Human milk (n=7)	4.3	2.2-8.8

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Sensitivity

The minimum detectable dose of human APOB is 0.68 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, cell culture supernatants samples were spiked with high concentrations of human APOB in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Serum and human milk were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.(The serum samples were initially diluted 1:10,000, the human milk samples were initially diluted 1:25)

		Human serum (Sample Diluent PT 1-ec)	Cell culture supernatants (Sample Diluent PT 1-ec)	Human milk (Sample Diluent PT 3)
1:2	Average% of Expected	100	106	100
	Range (%)	-	101-116	-
1:4	Average% of Expected	94	104	98
	Range (%)	86-104	99-108	92-103
1:8	Average% of Expected	91	98	101
	Range (%)	79-105	91-102	94-107
1:16	Average% of Expected	90	93	97
	Range (%)	81-113	83-104	76-118

References

1. Whayne TF. et al. (1981). Atherosclerosis. 1981 Jun;39(3):411-24.
2. Walldius G. et al. (2004). Clin Chem Lab Med. 2004;42(12):1355-63.

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