

Human Leptin Sandwich ELISA Kit Datasheet

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

General Information

Catalogue Number	KE00095
Product Name	Human Leptin Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6 - 500 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3952
SwissProt	P41159

Kit Components & Storage

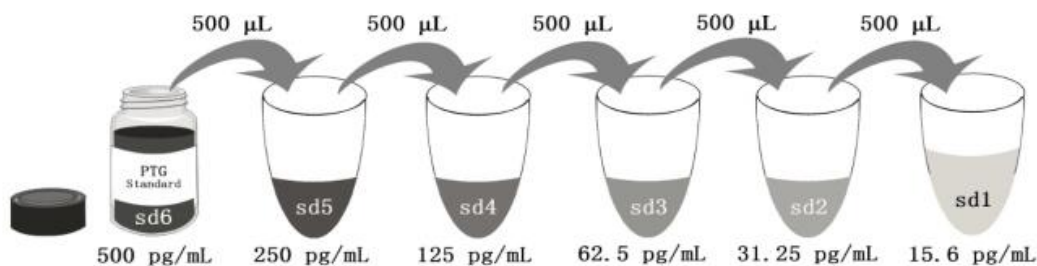
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 500 pg/bottle; lyophilized*	2 bottles	
Detection Antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle	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 1-ef is for protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 1 mL Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 500 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ef	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00095 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The human leptin ELISA kit is to be used to detect and quantify protein levels of endogenous human . The assay recognizes human leptin. An antibody specific for human leptin has been pre-coated onto the microwells. The human leptin protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human leptin is added to detect the captured human leptin 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

Leptin is a 16 kDa adipocyte-derived hormone, and it has attracted interest in the field of obesity research due to its role in the regulation of energy balance. Leptin, secreted from adipose tissues in proportion to the fat store, acts in the hypothalamus to regulate feeding behavior. leptin has also been shown to play an essential role in tumorigenesis, tumor progression, and metastasis, due to its oncogenic, mitogenic, pro-inflammatory, and pro-angiogenic actions. Mutations in this gene and/or its regulatory regions cause severe obesity, and morbid obesity with hypogonadism. Leptin has also been linked to type 2 diabetes mellitus development.

Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:50 is

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suggested according to the individual samples.

Safety Notes

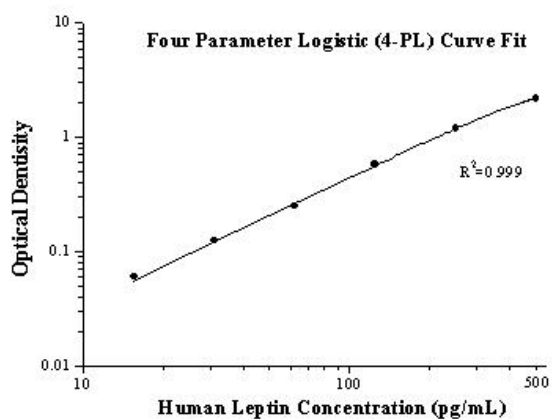
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.017 0.017	0.017	-
15.6	0.068 0.086	0.077	0.06
31.3	0.14 0.141	0.141	0.124
62.5	0.277 0.258	0.268	0.251
125	0.591 0.59	0.591	0.574
250	1.226 1.183	1.205	1.188
500	2.2 2.227	2.214	2.197

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	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	79.7	2.8	3.5	1	24	84.3	1.9	2.2
2	20	182.7	5.8	3.2	2	24	185.1	4.6	2.5
3	20	461.7	20.9	4.5	3	24	461.7	15.4	3.3

Recovery

The recovery of leptin 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 plasma	1:50	96	89 - 100
	1:100	92	82 - 113
Cell culture supernatants	1:2	97	87 - 111
	1:4	98	80-117

Sample Values

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

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum (n=32)	6,192	1,276 - 19,652

Sensitivity

The minimum detectable dose of human leptin is 2.0 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, human plasma samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatants were spiked with high concentrations of leptin 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 1:5)

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	87	104
	Range (%)	86 - 88	94 - 114
1:4	Average% of Expected	100	102
	Range (%)	98-101	91 - 114
1:8	Average% of Expected	97	99
	Range (%)	93 - 101	90 - 109
1:16	Average% of Expected	95	102
	Range (%)	89 - 102	94 - 111

References

1. Arnold M. et al. (2016) Cancer Epidemiol. 41:8-15.
2. Zhang Y. et al. (1994) Nature. 37:425-32.
3. de Luis DA. et al. (2009) Minerva Med. 229-36.
4. Jiang N. et al. (2014) Drug Des Devel Ther. 8:2295-302.
5. Vansaun MN. et al. (2013) Clin Cancer Res. 19:1926-1932.