

Human Adiponectin Sandwich ELISA Kit Datasheet

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

General Information

Catalogue Number	KE00080
Product Name	Human Adiponectin Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	62.5-4000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	9370
SwissProt	Q15848

Kit Components & Storage

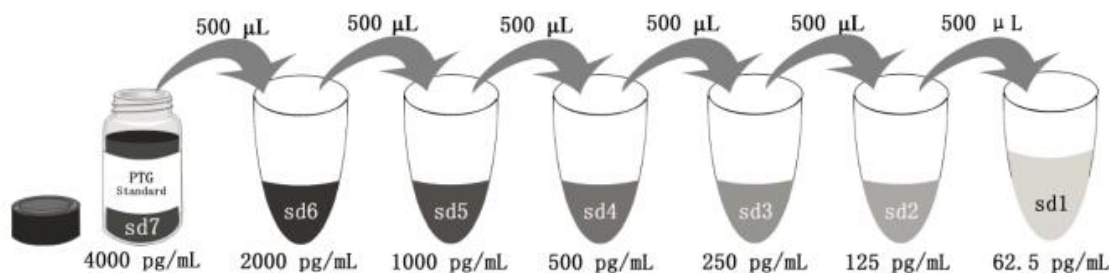
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 8000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µ L/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µ L/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 2 mL Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 4000 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 PT 1-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00080 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Adiponectin ELISA kit is to be used to detect and quantify protein levels of endogenous Adiponectin. The assay recognizes human total Adiponectin (low, middle, and high molecular weight). An antibody specific for Adiponectin has been pre-coated onto the microwells. The Adiponectin protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for Adiponectin is added to detect the captured Adiponectin 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

Adiponectin (AdipoQ), an adipocyte-derived hormone, is one of the most abundant adipokines in the blood circulation. Human adiponectin exists in three multimer forms, high-molecular-weight (HMW), middle-molecular-weight (MMW), and low-molecular-weight (LMW). Adiponectin modulates a number of metabolic processes, including improving insulin sensitivity and anti-inflammatory activity. The role of Adiponectin in reproduction is not yet fully understood, but the expression of Adiponectin in reproductive tissues has been observed in various animals and humans, including chicken testis, bovine ovary, and human placenta. Adiponectin exerts its effects by activating a range of different signaling molecules via binding to two transmembrane Adiponectin receptors, AdipoR1 and AdipoR2. AdipoR1 is expressed primarily in the skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. Adiponectin May play a role in cell growth, angiogenesis and tissue remodeling by binding and sequestering various growth factors.

Sample Preparation

The samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2 is suggested for cell supernates. The serum and plasma samples were initially diluted 1:2,000.

Safety Notes

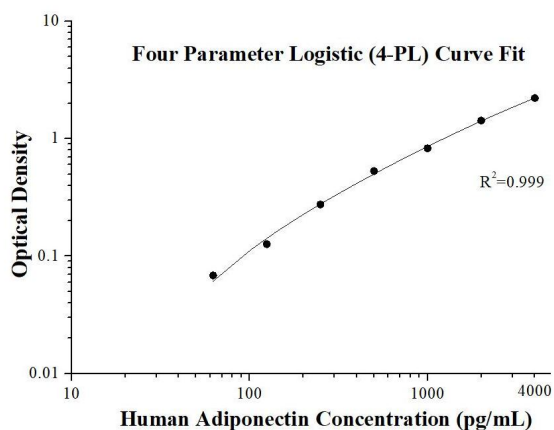
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.024 0.026	0.025	-
62.5	0.084 0.103	0.094	0.069
125	0.159 0.144	0.152	0.127
250	0.316 0.284	0.3	0.275
500	0.572 0.538	0.555	0.53
1000	0.852 0.856	0.854	0.829
2000	1.402 1.5	1.451	1.426
4000	2.233 2.254	2.244	2.219

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	829.0	76.7	9.3	1	24	681.7	58.0	8.5
2	20	1,708.0	164.5	9.6	2	24	1,421.9	138.0	9.7
3	20	2,354.9	158.3	6.7	3	24	2,467.6	156.2	6.3

Recovery

The recovery of Adiponectin 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	1:10,000	90	69-116
	1:20,000	100	95-104
Cell culture supernatants	1:2	105	95-115
	1:4	94	81-104

Sample Values

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

Sample Type	Mean of Detectable (ug/mL)	Range (ug/mL)
Human plasma (n=24)	6.6	6.2-13.0

Sensitivity

The minimum detectable dose of human Adiponectin is 24.0 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 serum and 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 Adiponectin in matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The serum and plasma were initially diluted 1:2,000)

		Human Serum	Human Plasma	Cell culture supernatants
1:2	Average% of Expected	107	111	111
	Range (%)	99-114	110-113	109-114
1:4	Average% of Expected	99	113	105
	Range (%)	93-104	111-116	103-107
1:8	Average% of Expected	90	114	98
	Range (%)	86-95	109-120	96-99
1:16	Average% of Expected	83	108	100
	Range (%)	81-85	104-112	92-109

References

1. Y. Arita. et al. (1999). *Biochem Biophys Res Commun.* 257: 79-83.
2. T. Kadowaki. et al. (2005). *Endocr Rev.*26: 439-451.
3. H. Tilg. et al. (2005). *Expert Opin Ther Targets.* 9: 245-251.
4. T. Yamauchi. et al.(2003). *Nature.* 423: 762-769.