

## Mouse Adiponectin sandwich ELISA kit datasheet

For the quantitative detection of mouse Adiponectin in serum, plasma, cell culture supernatants and tissue homogenates.

### general information

Catalogue Number	KE10044
Product Name	Mouse Adiponectin ELISA Kit
Species cross-reactivity	Mouse Adiponectin
Range (calibration Range)	31.25 - 2000 pg/mL
Tested applications	Quantification ELISA

### database links

Entrez Gene	11450 (Mouse)
SwissProt	Q60994 (Mouse)

### 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 - 4000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-HRP (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1 - 30 mL/bottle. For serum and plasma samples and tissue homogenates.	2 bottles	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernatants	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	

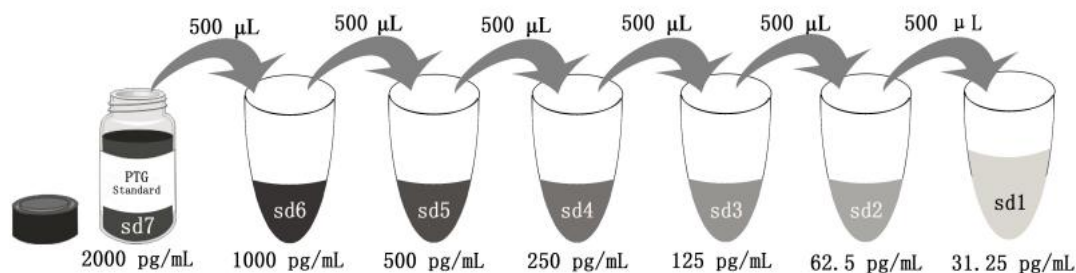
**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 1 is for protein standard, serum, plasma and tissue homogenates.

Sample Diluent PT 1-ef is for protein standard, cell culture supernatants.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 2 mL Sample Diluent PT 1 or PT 1-ef in protein standard. This reconstitution gives a stock solution of 2000 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 or 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

KE10044 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 mouse Adiponectin. 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, also known as Acrp30, AdipoQ, and GBP28, is an adipocyte-secreted protein that circulates in high concentrations in the serum and acts to increase insulin sensitivity. Adiponectin modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation. The serum concentration of adiponectin decreases in obese patients, opposite to other adipocytokines. In recent studies, serum adiponectin level progressively decreased in patients according to the severity of obesity, insulin resistance, diabetes mellitus and cardiovascular diseases. Low serum adiponectin level may be a predictive factor for type 2 diabetes mellitus (T2DM) and other cardiovascular diseases.

## sample preparation

Samples may require proper dilution to fall within the range of the assay. Mouse serum or plasma is better to be diluted 1:20,000 or 1:40,000 before assay. Cell culture supernatants is better to be diluted 1:2 or 1:4 before assay.

## safety notes

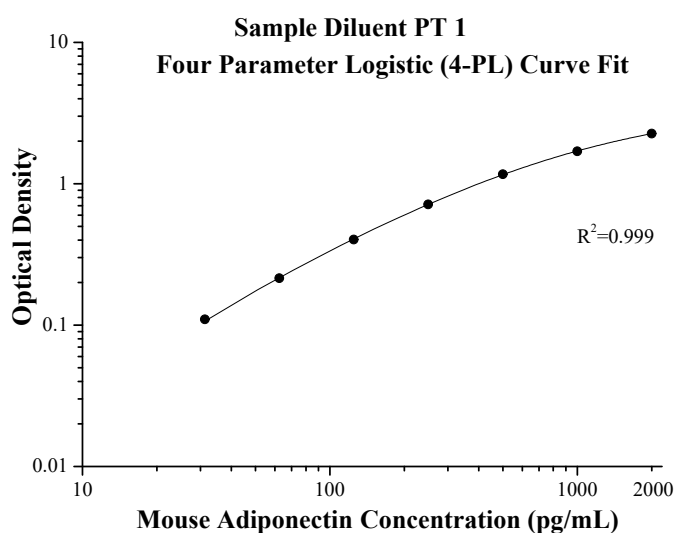
This product is sold for lab research and development use ONLY and not for use in humans or animals.  
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## assay procedure summary

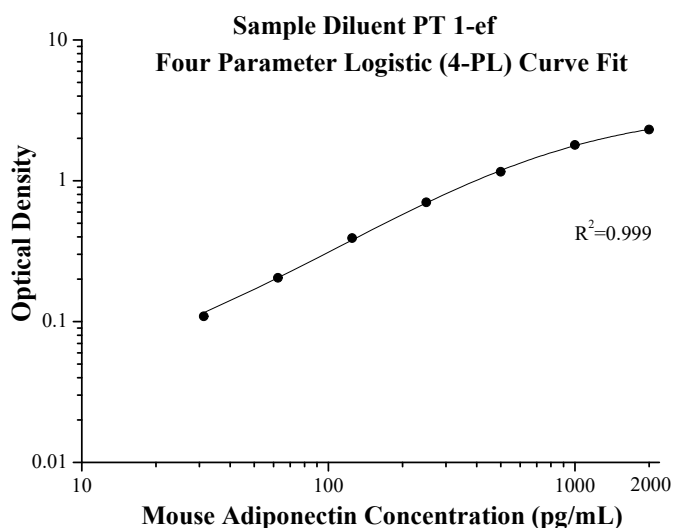
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	<b>120 min</b>	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 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.



(pg/mL)	O.D	Average	Corrected
0	0.043	0.044	-
	0.044		
31.25	0.153	0.153	0.110
	0.153		
62.5	0.259	0.258	0.215
	0.257		
125	0.454	0.447	0.403
	0.439		
250	0.771	0.756	0.713
	0.741		
500	1.228	1.213	1.169
	1.197		
1000	1.748	1.740	1.697
	1.732		
2000	2.346	2.307	2.264
	2.268		



(pg/mL)	O.D	Average	Corrected
0	0.060	0.057	-
	0.053		
31.25	0.169	0.165	0.109
	0.161		
62.5	0.263	0.261	0.204
	0.258		
125	0.432	0.448	0.392
	0.464		
250	0.752	0.759	0.702
	0.765		
500	1.198	1.214	1.158
	1.230		
1000	1.876	1.853	1.796
	1.829		
2000	2.398	2.369	2.312
	2.339		

## 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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	940.5	224.3	55.5	1,009.9	237.4	56.5
SD	43.0	8.3	2.9	79.9	10.2	3.1
CV%	4.6	3.7	5.2	7.9	4.3	5.5

## recovery

The recovery of Adiponectin spiked to three different levels in four samples throughout the range of the assay in Plasma was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:80,000	109	100-122
	1:160,000	95	91-101
Mouse fat tissue homogenates	1:4,000	101	97-106
	1:8,000	91	82-95
Cell culture supernatants	1:4	90	77-104
	1:8	96	89-102

## sample values

Sample Type	Mean (ng/mL)	Range (ng/mL)
Mouse serum (n=19)	11,009	4,444-16,755

Cell Culture Supernatants - lungs from one mouse were chopped into 1-2 mm pieces and seeded into approximately 30 mL of media containing RPMI, 10% fetal bovine serum, 50  $\mu$  M  $\beta$ -mercaptoethanol, and L-glutamine. An aliquot of the cell culture supernatants was removed for evaluation, assayed for levels of mouse Adiponectin, and measured 584 pg/mL.

Tissue Homogenates - Organs from 2 mice were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized in 5-10 mL of PBS in a tissue homogenizer, and stored at  $\leq -80^{\circ}\text{C}$  5 min. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g to remove particulate. Homogenates from spleen, liver, and fat tissue were assayed for mouse Adiponectin and measured 324 ng/mL, 10.4 ng/mL, and 540 ng/mL, respectively.

## sensitivity

The minimum detectable dose of mouse Adiponectin is 6.9 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, samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The mouse serum samples were initially diluted 1:20,000. The fat tissue homogenates samples were initially diluted 1:1,000. Cell culture supernatants were initially diluted 1:2)

		Mouse serum (PT 1)	Mouse fat tissue homogenates (PT 1)	Cell culture supernatants (PT 1-ef)
1:2	Average% of Expected	100	100	100
	Range (%)	-	-	-
1:4	Average% of Expected	103	98	107
	Range (%)	100-107	95-100	104-111
1:8	Average% of Expected	102	102	112
	Range (%)	97-106	96-107	104-123
1:16	Average% of Expected	110	100	
	Range (%)	104-113	96-104	

## references

1. Wang ZV. et al. (2016) J Mol Cell Biol. 8(2):93-100.
2. Liu M. et al. (2014) Best Pract Res Clin Endocrinol Metab.28(1):25-31.
3. Díez JJ. et al. (2003) Eur J Endocrinol.148(3):293-300.
4. Yamauchi T. et al. (2003) Nature.423(6941):762-9.