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HUMAN ADIPONECTIN HIGH SENSITIVITY ENZYME IMMUNOASSAY KIT

catalogue # A05186

96 wells

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For research laboratory use only.
Not for diagnostic use.

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HUMAN ADIPONECTIN HIGH SENSITIVITY EIA KIT

96 wells
Storage: 2-8℃
Expiry date: stated on the package

This kit contains:

- ☞A covered 96 well plate, pre-coated with an anti-Adiponectin antibody, ready to use
- ☞One vial of anti-Adiponectin tracer, ready to use
- ☞Eight vials of human Adiponectin standards (1, 2, 5, 10, 20, 50, 100 and 150 ng/mL), ready to use
- ☞One vial of Substrate (TMB) solution, ready to use
- ☞One vial of Stop solution (0.2 M H_2SO_4), ready to use
- ☞One vial of concentrated EIA Buffer (10x), liquid
- ☞Two vials of Quality Controls: low and high, diluted 30x, liquid
- ☞One vial of concentrated Wash buffer (10x), liquid
- ☞One instruction booklet
- ☞One template sheet
- ☞One well cover sheet

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 41 samples in duplicate.

PRECAUTIONS FOR USE

Users are recommended to read all instructions for use before starting work.

Each time a new pipet tip is used, aspirate a sample of reagent and dispense into the same vessel. Repeat this operation two or three times before distribution.

For research laboratory use only.
Not for diagnostic use.
Do not pipet liquids by mouth.
Do not use kit components beyond the expiration date.
Do not mix different lot numbers.
Do not eat, drink, or smoke in area in which kit reagents are handled.
Avoid splashing.

This kit contains components of human origin. These materials were found non-reactive for HbsAg, HCV antibody and for HIV 1/2 antibody and antigen. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents. Wear gloves and laboratory coats are recommended when handling immunodiagnostics materials and samples of human origin.

Stop solution and Substrate solution are potential harmful solution. To avoid any contact, wear eye, hand, face and clothing protection when handling these reagents.

PRINCIPLE OF THE ASSAY

Adiponectin, also referred to as Acrp30, AdipoQ and GBP-28, is an 244 aminoacid protein, which is physiologically active, specifically and highly expressed in adipose cells (adipokine). Adiponectin forms homotrimers, which are the building blocks for higher order complexes found circulating in serum.

Paradoxically, adipose tissue-expressed adiponectin levels are inversely related to the degree of adiposity. A reduction in adiponectin serum levels is accompanied by insulin resistance states, such as obesity and type II diabetes mellitus. Adiponectin has been shown to increase insulin sensitivity and decrease plasma glucose by increasing tissue fat oxidation. It inhibits the inflammatory processes of atherosclerosis

suppressing the expression of adhesion and cytokine molecules in vascular endothelial cells and macrophages, respectively.

This Enzyme Immunometric Assay (EIA) is based on a double-antibody sandwich technique. The wells of the plate supplied with the kit are coated with a polyclonal antibody specific of human adiponectin. This antibody will bind any human adiponectin introduced in the wells (sample or standard). An horseradish peroxydase (HRP) conjugated polyclonal antibody which binds selectively to different epitopes on the adiponectin molecule, is also added to the wells.

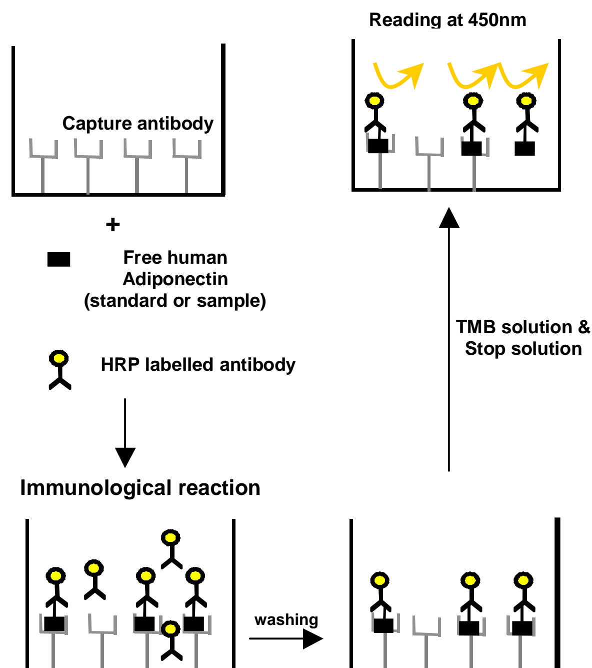
This allows the two antibodies to form a sandwich by binding on different parts of the human adiponectin molecule.

The sandwich is immobilised on the plate so the excess reagents may be washed away. The concentration of the human adiponectin is then determined by measuring the enzymatic activity of the HRP using the hydrogen peroxide/TMB solution. The reaction is stopped by addition of sulfuric acid solution. The HRP tracer acts on TMB Reagent to form a yellow compound.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of the human adiponectin present in the well during the immunological incubation.

Note that this kit allows you to detect Low molecular weight and High Molecular Weight polymers of adiponectin, but was not able to detect trimer of adiponectin.

The principle of the assay is summarised below:



MATERIALS AND EQUIPMENT REQUIRED

In addition to standard laboratory equipment, the following material is required:

FOR THE ASSAY

☞Precision micropipettes (5 to 1000 µL)

☞Spectrophotometer plate reader (450 nm +/- 10 nm filter)

☞Microtitration washer (or washbottles)

☞Microplate shaker

✐Multichannel pipette 100 µL and disposable tips

✐Distilled or deionised water

✐Polypropylene tubes

SAMPLE PREPARATION

This assay may be used to measure human Adiponectin in human samples such as serum, plasma, breast milk, urine and tissue culture supernatant.

Adiponectin levels are significantly lower (2-3 times) in breast milk or urine than in serum and plasma. Therefore the protocols are slightly different.

GENERAL PRECAUTIONS

✐All samples must be free of organic solvents prior to assay.

✐Samples should be assayed immediately after collection or should be stored at -80℃.

SERUM AND PLASMA

Dilute samples 1/300 in EIA buffer preferably in two steps: add 10 µL sample to 90 µL EIA buffer (dilution 10x). Mix well. Add 10 µL of this dilution 10x to 290 µL EIA buffer for the final dilution. Mix well.
Serum or plasma samples should be stored frozen (preferably at -80 ℃, then the stability is at least 1 year). It has been proved that the adiponectin concentration in serum or plasma samples does not decrease after three thawing-freezing cycles. Nevertheless, repeated thawing-freezing should be avoided.

Undiluted samples are stable at least 1 week at 2-8℃ or 1 day at room temperature.
Diluted samples have to be stored frozen.

BREAST MILK AND URINE SAMPLES

Dilute samples 1/3 in EIA buffer (i.e. 100 µL sample + 200 µL EIA buffer).
Stability of milk and urine samples have not been tested.

REAGENT PREPARATION

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready to use, except the Quality Control, EIA buffer and wash buffer.

✐EIA buffer

Reconstitute one vial of concentrated EIA buffer (20 mL, 10x) to 200 mL with 180 mL distilled or deionised water.
Stability at 2-8℃: 1 month.

✐Quality Controls

Quality Controls are supplied diluted 30x. In order to get them diluted 300x, dilute QC 1/10 in EIA buffer : add 30 µL QC to 270 µL EIA buffer (dilution 10x). Mix thoroughly by gentle inversion, vortex is recommended.

✐Wash buffer

Reconstitute one vial of concentrated Wash buffer (100 mL, 10x) to 900 mL with distilled or deionised water.

✐Hydrogen peroxide/TMB solution

Substrate solution should remain colourless until added to the plate. Keep substrate solution protected from the light.

ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

DISTRIBUTION OF REAGENTS AND SAMPLES

A plate set-up is suggested on this page. The content of each well may be recorded on the sheet provided with the kit.

PIPETTING THE REAGENTS

All samples and reagents must reach room temperature prior performing the assay. Use different tips to pipet the buffer, standard, sample, tracer antiserum and other reagents.

➯ Human Adiponectin standard:

Dispense 100 µL of each diluted standard (5 to 150 ng/mL for serum or plasma samples and 1 to 50 ng/mL for milk or urine samples) in duplicate to appropriate wells. Start with the lowest concentration standard and equilibrate the tip in the next higher standard before pipetting.

➯ Quality Control and samples:

Dispense 100 µL of diluted Quality Control and samples in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA buffer.

	A	B	C	D	E	F	G	H
12	*	*	*	*	*	*	*	*
11	*	*	*	*	*	*	*	*
10	*	*	*	*	*	*	*	*
9	*	*	*	*	*	*	*	*
8	*	*	*	*	*	*	*	*
7	*	*	*	*	*	*	*	*
6	*	*	*	*	*	*	*	*
5	*	*	*	*	*	*	*	*
4	*	*	*	*	*	*	*	*
3	*	*	*	*	*	*	*	*
2	S4	S4	S5	S5	S6	S6	*	*
1	B	B	S1	S1	S2	S2	S3	S3

B: Blank
S1-S6: Standards 1-6
*: Samples or Quality Controls

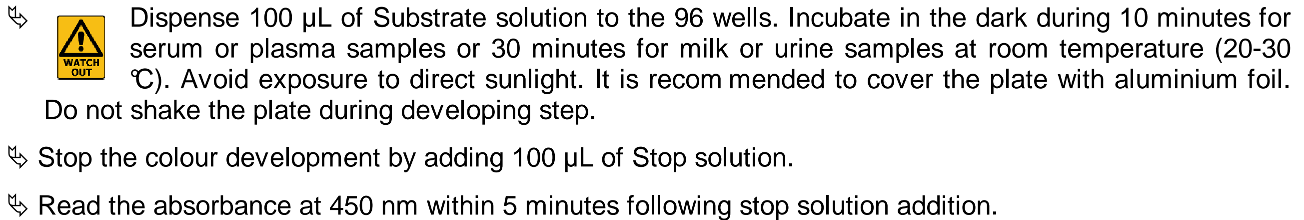
INCUBATING THE PLATE

➯ Incubate the plate for 1 hour, shaking at 300 rpm on an orbital microplate shaker.

➯ Rinse the wells 3 times with the Wash buffer (350 µL per well). After the final wash, invert and tap the plate vigorously against paper towel.

- Anti-adiponectin antibody HRP tracer:
  Dispense 100 µL to each well, except the Blank (B) wells. Mix gently.
- Incubate the plate for 1 hour, shaking at 300 rpm on an orbital microplate shaker.
- Rinse the wells 3 times with the wash buffer (350 µL per well). After the final wash, invert and tap the plate vigorously against paper towel.

DEVELOPING AND READING THE PLATE



- Dispense 100 µL of Substrate solution to the 96 wells. Incubate in the dark during 10 minutes for serum or plasma samples or 30 minutes for milk or urine samples at room temperature (20-30 °C). Avoid exposure to direct sunlight. It is recommended to cover the plate with aluminium foil. Do not shake the plate during developing step.
- Stop the colour development by adding 100 µL of Stop solution.
- Read the absorbance at 450 nm within 5 minutes following stop solution addition.

  Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the lowest standard (the highest absorbance of the calibration curve), perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine Adiponectin concentration of off-scale samples. ***The readings at 405 nm should not replace the on-scale readings at 450 nm.***

Enzyme Immunoassay Protocol (Volumes are in µL)			
	Blank	Standard	Sample
Standard	-	100	-
Sample	-	-	100
Incubate the plate at room temperature for 1 hour			
Wash the plate 3 times			
Tracer	-	100	100
Incubate the plate at room temperature for 1 hour			
Wash the plate 3 times			
TMB solution	100	100	100
Incubate the plate in the dark at room temperature during 10 minutes for serum or plasma samples ; during 30 minutes for milk or urine sample			
Stop solution	100	100	100
Read the plate at 450 nm			

DATA ANALYSIS

Make sure that your plate reader has subtracted the absorbance readings of the blank well (absorbance of TMB solution) from the absorbance readings of the rest of the plate. If not, do it now.

- Using a logit-log graph paper, plot the absorbance for each standard (y axis) versus concentration (x axis) of standards. Draw a best-fit line through the points.

- To determine the concentration of your samples, find the absorbance value on the y axis. Read the corresponding value on the x axis which is the concentration of your diluted QCs and diluted unknown sample.
  Since Quality Controls and samples have been diluted before the assay, the measured concentration of Quality Controls calculated from the standard curve must be multiplied by a dilution factor of 300, and the measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor.

- Most plate readers are supplied with curve-fitting software capable of graphing this type of data (logit/log or 4-parameter). If you have this type of software, we recommend using it. Refer to it for further information.

TYPICAL DATA

EXAMPLE DATA

The following data are for demonstration purposes only. Your data may be different but still be correct. These data were obtained using all reagents as supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Human Adiponectin (10 minutes Substrate Incubation)	mAU
Blank	22
Standard 150 ng/mL	3 052
Standard 100 ng/mL	2 583
Standard 50 ng/mL	1 661
Standard 20 ng/mL	765
Standard 10 ng/mL	394
Standard 5 ng/mL	203
QC High	2 029
QC Low	623

Human Adiponectin (30 minutes Substrate Incubation)	mAU
Blank	11
Standard 50 ng/mL	2 982
Standard 20 ng/mL	1 536
Standard 10 ng/mL	844
Standard 5 ng/mL	439
Standard 2 ng/mL	185
Standard 1 ng/mL	118
QC Low	623

ACCEPTABLE RANGE

☞QC samples: see label on the vials.

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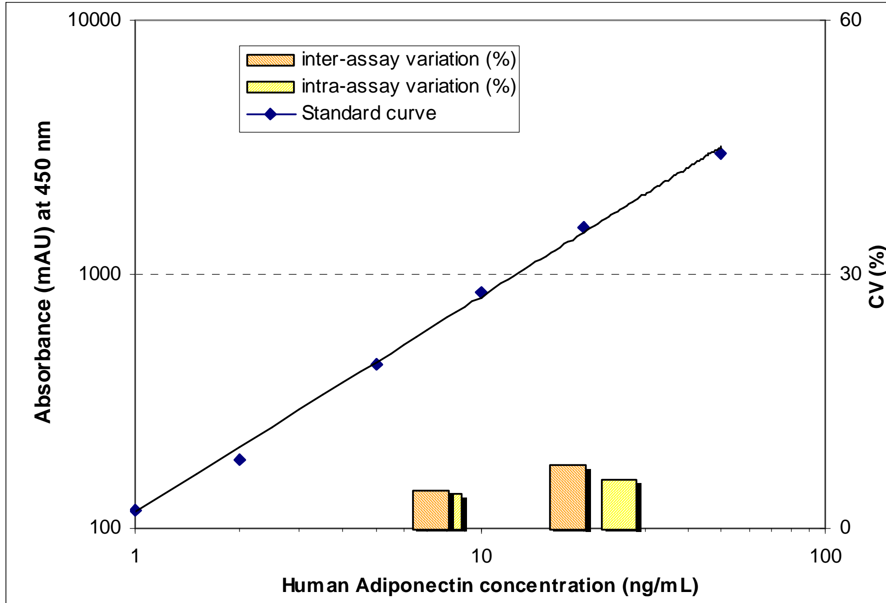
HUMAN ADIPONECTIN HIGH SENSITIVE STANDARD CURVE



SPI bio

ASSAY VALIDATION AND CHARACTERISTICS

The Enzyme Immunometric assay of human Adiponectin has been validated for its use in human serum, plasma breast milk urine samples and tissue culture supernatant.

☞Cross-reactivity:

The assay recognizes human and recombinant Adiponectin.

- Human leptin <0.1%
- Human resistin <0.1%
- Human leptin receptor <0.1%
- Sheep Adiponectin <0.1%
- Goat Adiponectin <0.1%
- Horse Adiponectin <0.1%
- Cow Adiponectin <0.1%
- Pig Adiponectin <0.1%
- Rabbit Adiponectin <0.1%
- Monkey serum equivalent to 15-19 µg/mL

☞Sensitivity:

The limit of detection (defined as such a concentration of human Adiponectin giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + $3*SD_{blank}$) is better than 0.5 ng/mL of sample. The EIA buffer was pipetted into blank wells, and the microtiter plate is blanked on air.

☞Precision:

- Intra-assay (n=8)

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	8.34	0.35	4.1
2	23.99	1.12	4.7

- Inter-assay (Run-to-Run, n=9)

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	7.57	0.24	4.0
2	19.32	1.14	7.4

☞Recovery test:

Sample	Observed (µg/mL)	Expected (µg/mL)	Recovery O/E (%)
1	3.21	-	-
	7.92	8.21	96
	12.58	13.21	96
	21.12	23.21	91
2	5.60	-	-
	10.22	10.60	96
	15.87	15.60	102
	24.91	25.60	97

☞Dilution test:

Sample	Dilution	Observed (µg/mL)	Expected (µg/mL)	Recovery O/E (%)
1	-	17.12	-	-
	1:2	8.51	8.56	100
	1:4	4.29	4.28	102
	1:8	2.21	2.14	103
2	-	12.61	-	-
	1:2	6.50	6.31	104
	1:4	3.28	3.15	106
	1:8	1.60	1.58	102

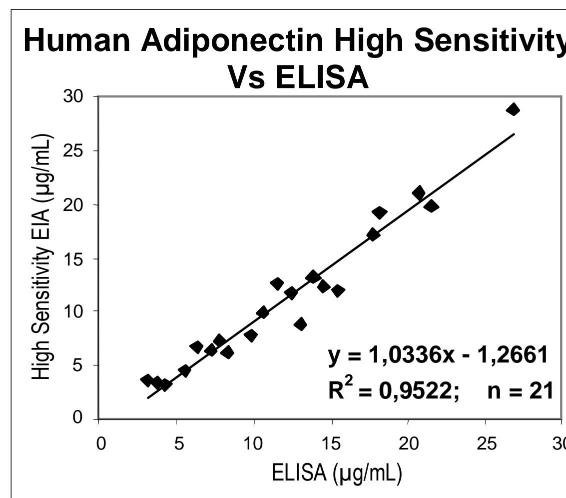
☞Serum/ Plasma Samples:

Citrate, EDTA and heparin plasmas were compared to respective serum samples obtained from healthy persons (n = 4) in the same time.

Sample (n = 4)	Mean Adiponectin (µg/mL)	Plasma /Serum (%)
Serum	9.31	-
Citrate Plasma	8.22	88.2
EDTA Plasma	8.80	94.4
Heparin Plasma	9.67	103.8

☞Method comparison:

We have compared the human Adiponectin ELISA (#A05185) with our High Sensitivity EIA (#A05186) on 21 serum samples. The correlation graph was obtained.



ASSAY TROUBLE SHOOTING

☞Absorbance values too low:

- One reagent has not been dispensed
- Incorrect preparation or reagent storage
- Assay performed before reagents reach room temperature

☞High signal and background in all wells:

- Inefficient washing
- Overdeveloping; incubation time should be reduced before adding Stop Solution

☞High dispersion of duplicates:

- Poor pipetting technique or irregular plate washing.

These are a few examples of problems that may occur. If you need further assistance, SPI-BIO will be happy to answer any questions or information about this assay. Please feel free to contact our technical support staff by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (sales@spibio.com), and be sure to indicate the lot number of the kit (see outside of the box).

SPI-BIO offers a training workshop in EIA practice & theory. This workshop is given twice a year. For further information, please contact our Customer Relation Representative (33 (0)1 39 30 62 60).

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