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HUMAN ADIPONECTIN ENZYME IMMUNOASSAY KIT

catalogue # A05185

96 wells

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*For research laboratory use only.
Not for diagnostic use.*



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HUMAN ADIPONECTIN EIA KIT

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

This kit contains:

- ☞ A covered 96 well plate, pre-coated with recombinant human Adiponectin, ready to use
- ☞ One vial of anti-Adiponectin tracer, ready to use
- ☞ Seven vials of human Adiponectin standards (0.1, 0.2, 0.5, 1, 2, 5 and 10 µg/mL), ready to use
- ☞ Two vials of Substrate (TMB) solution, ready to use
- ☞ One vial of Stop solution (0.2 M H₂SO₄), ready to use
- ☞ Two vials of Quality Controls: low and high, liquid
- ☞ Two vials of EIA buffer, ready to use
- ☞ 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 40 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 immunodiagnosics 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.

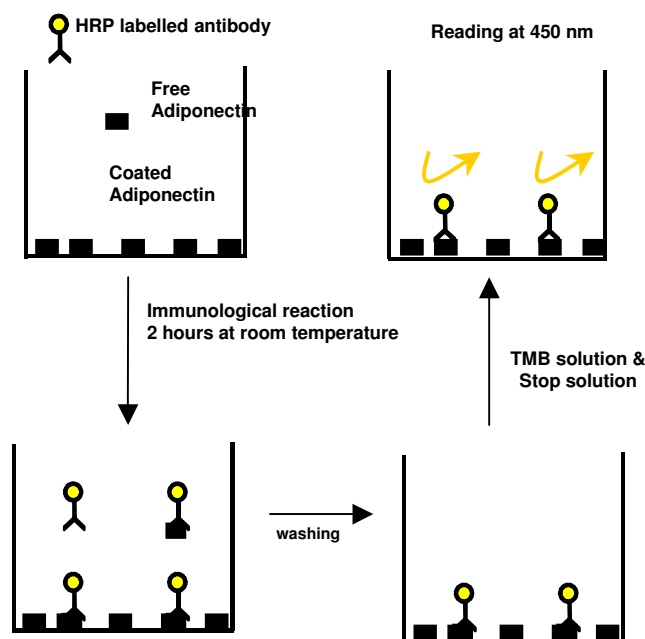
This Enzyme Linked ImmunoSorbent Assay (ELISA) is based on the competition between free adiponectin and coated adiponectin, in presence of a known quantity of HRP labelled adiponectin antibody (tracer).

The plate is washed to remove any unbound reagent, and the hydrogen peroxide/TMB substrate is added to the wells. The HRP tracer acts on the hydrogen peroxide/TMB substrate to form a yellow compound that absorbs at 450 nm.

The reaction is stopped by addition of sulfuric acid solution.

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

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 (10 to 1000 μL)
- ☞ Spectrophotometer plate reader (450 nm \pm 10 nm filter)
- ☞ Microtitration washer (or washbottles)
- ☞ Microplate shaker
- ☞ Multichannel pipette 50-200 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 and plasma.

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°C.

SERUM AND PLASMA

- ☞ Samples
 - No prior extraction procedure is necessary.
 - To measure human Adiponectin, dilute samples 1/30 in EIA buffer (i.e. 10 µL sample + 290 µL EIA buffer)
- ☞ Storage and stability of samples
 - Serum or plasma samples should be stored frozen (preferably at -80°C, then the stability is at least 1 year). Repeated thawing-freezing cycles should be avoided.
 - Undiluted samples are stable at least 1 week at 2-8°C or 1 day at room temperature.
 - Diluted samples have to be stored frozen.

REAGENT PREPARATION

Assay reagents are supplied ready to use, except the concentrated Wash buffer.

- ☞ Human Adiponectin standard
 - Dilute standards 1/3 in EIA buffer prior to using (i.e. 50 µL standard + 100 µL EIA buffer).
- ☞ Quality Controls
 - Quality Controls are ready to use: supplied diluted 1/30. Do not dilute prior the use.
- ☞ Wash buffer
 - Dilute one vial of concentrated Wash buffer (100mL, 10x) to 1000 mL with distilled or deionised water.
 - Stability at 2-8°C: 1 month
- ☞ 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 the following page. The contents 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 to performing the assay. Use different tips to pipet the buffer, standard, sample, tracer and other reagents.

- ☞ Human Adiponectin standard:
 - Dispense 50 µL of the seven standards (S1 to S7), in duplicate to appropriate wells. Start with the lowest concentration standard and equilibrate the tip in the next higher standard before pipetting.

Enzyme Immunoassay Protocol (Volumes are in μL)			
	Blank	Standard	Sample
Standard	-	50	-
Sample	-	-	50
Tracer	-	50	50
Incubate at room temperature during 2 hours			
Wash the plate 3 times			
TMB solution	200	200	200
Incubate the plate in the dark at room temperature during 10-15 minutes			
Stop solution	50	50	50
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 semi-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 unknown sample.
- ↪ As the standards are to be diluted 3-times, while the samples 30-times, the values calculated from the calibration curve have to be multiplied by a dilution factor of 10 to obtain the true results!
- ↪ 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

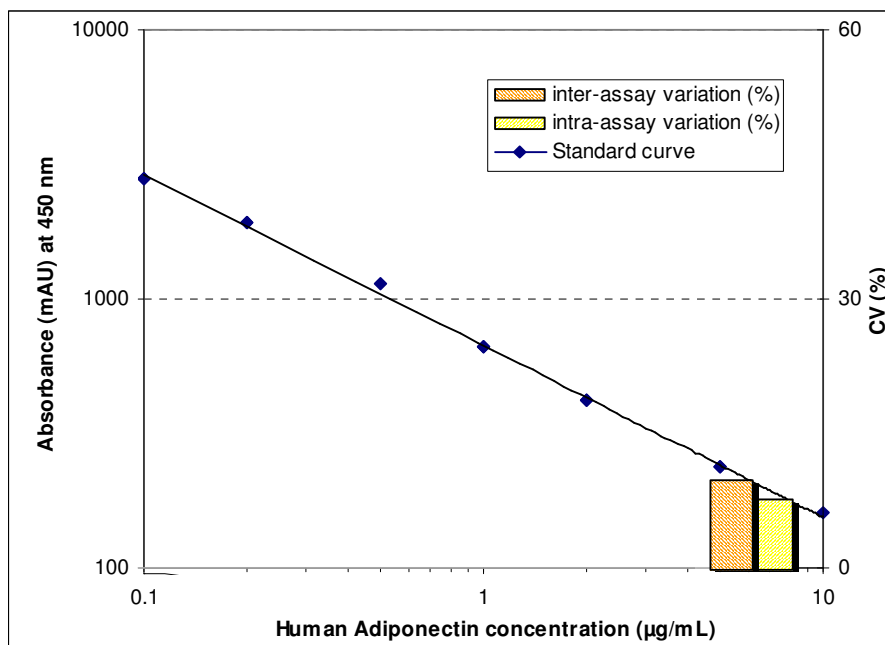
The following data are for demonstration purposes only. Your data may be different but still be correct. These data were obtained using all reagents as supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Human Adiponectin	mAU
Standard 10 µg/mL	160
Standard 5 µg/mL	236
Standard 2 µg/mL	418
Standard 1 µg/mL	661
Standard 0.5 µg/mL	1 136
Standard 0.2 µg/mL	1 935
Standard 0.1 µg/mL	2 788
QC High	381
QC Low	807

ACCEPTABLE RANGE

☞ QC samples: see label on the vials.

HUMAN ADIPONECTIN STANDARD CURVE



ASSAY VALIDATION AND CHARACTERISTICS

The Enzyme Immunometric assay of human Adiponectin has been validated for its use in serum and human plasma.

☞ **Cross-reactivity:**

Human Leptin	<0.1 %
Human Leptin receptor	<0.1 %
Human Resistin	<0.1 %
Mouse Adiponectin	<0.1 %
Rabbit Adiponectin	<0.1 %
Cow Adiponectin	<0.1 %
Horse Adiponectin	<0.1 %

☞ No interference has been observed with hemoglobin (5 mg/mL), bilirubin mixed isomers (0.4 mg/mL) and triglycerides (0.25 mg/mL).

☞ **Sensitivity:**

The limit of detection calculated as the concentration of human Adiponectin corresponding to the blank average minus three standard deviations is 7 ng/mL.

☞ **Precision:**

• **Intra-assay variation (n=8)**

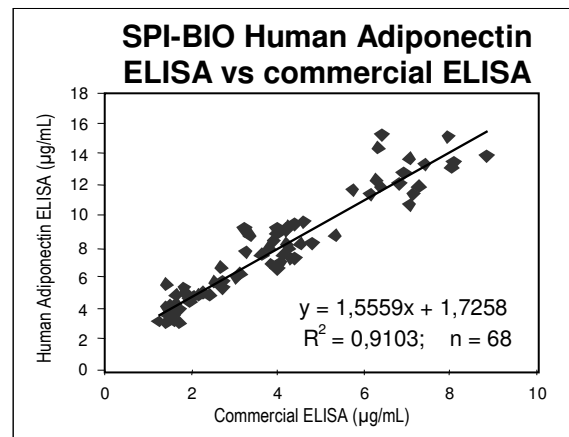
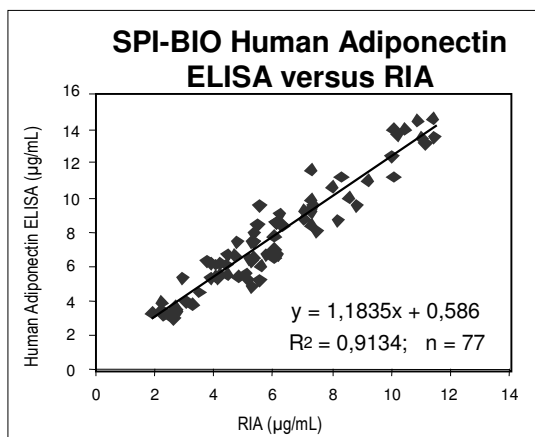
Sample	Mean (µg/mL)	Standard Deviation (µg/mL)	CV (%)
1	7.14	0.50	7.0
2	21.17	1.35	6.4

• **Inter-assay variation (n=8)**

Sample	Mean (µg/mL)	Standard Deviation (µg/mL)	CV (%)
1	5.27	0.43	8.2
2	17.78	1.29	7.3

☞ **Method comparison:**

The SPI-BIO's Human Adiponectin ELISA was compared to other commercial immunoassays, measuring 77 or 68 serum samples, in radioimmunoassay (RIA) or EIA, respectively, gave the following correlation graphs.



☞ Recovery test:

Sample	Observed (µg/mL)	Expected (µg/mL)	Recovery O/E (%)
1	4.36	-	-
	8.48	9.36	91
	12.25	14.36	85
	23.12	24.36	95
2	6.90	-	-
	12.29	11.90	103
	15.23	16.90	90
	25.83	26.90	96

☞ Dilution test:

Sample	Dilution	Observed (µg/mL)	Expected (µg/mL)	Recovery O/E (%)
1	-	14.79	-	-
	1:2	7.87	9.63	90
	1:4	3.56	4.82	89
	1:8	1.84	2.41	94
2	-	23.39	-	-
	1:2	11.50	11.70	98
	1:4	5.69	5.85	97
	1:8	2.95	2.92	101

☞ Serum/ Plasma samples:

Citrate, EDTA and heparin plasma were compared to respective serum samples obtained from healthy persons (n = 15) in the same time.

Sample (n = 15)	Mean Adiponectin (µg/mL)	Plasma/ Serum (%)
Serum	11.77	-
Citrate Plasma	10.17	86.3
EDTA Plasma	11.15	94.7
Heparin plasma	10.99	93.3

☞ Normal values:

The following results were obtained when serum samples from 335 healthy persons were analysed with SPI-BIO's Human Adiponectin EIA. It is highly recommended that each laboratory should draw their own values.

Gender	BMI* (kg/m ²)	n	Mean (µg/mL)	SD (µg/mL)
Men	< 25	41	10.9	4.0
	25-30	52	8.8	4.0
	> 30	23	8.3	2.8
	total	115	9.5	3.9
Women	< 25	92	13.6	5.4
	25-30	56	13.9	8.6
	> 30	57	11.4	3.8
	total	220	13.2	6.1

*BMI: Body Mass Index



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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