Adiponectin (Human)

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Adiponectin (Human)
ELISA kit
#A05185.96 wells

For research laboratory use only
Not for human diagnostic use

Fabriqué en France
Made in France

#A11185
Version: 0121

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96 wells
Storage: +4°C
Expiry date: stated on the package

This kit contains:

Designation	Item #	Quantity per kit	Form
Adiponectin (human) precoated Microtiter Plate	A08185	1	-
Adiponectin (human) Tracer	A04185	1	Liquid
Adiponectin (human) Standard (0.1, 0.2, 0.5, 1, 2, 5, 10 µg/mL)	A06185	7x1	Liquid
Adiponectin (human) Quality Control HIGH	A10185_H	1	Liquid
Adiponectin (human) Quality Control LOW	A10185_L	1	Liquid
ELISA Buffer	A07010	2	Liquid
Concentrated Wash Buffer (10x)	A17012	1	Liquid
Substrate Solution (TMB)	A09010	2	Liquid
Stop Solution	A22000	1	Liquid
Technical Booklet	A11185	1	-
Well cover Sheet	-	1	-

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 38 samples in duplicate.

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Precaution for use

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

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- Do not eat, drink or smoke in area where 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 materials and samples of human origin.
Stop Solution and Substrate Solution are harmful solutions. Wearing lab gloves, laboratory coat and eye protection glasses is recommended when assaying kit materials and samples.

Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), which is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

Background

Adiponectin, also referred to as Acrp30, AdipoQ and GBP-28, is a 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.

Principle of the assay

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 unbounded 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 an acidic 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.

Assay characteristics

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

Sensitivity

The limit of detection (LOD) (defined as concentration of analyte giving absorbance lower than mean absorbance of blank* minus three standard deviations of the absorbance of blank: $A_{blank} - 3xSD_{blank}$) is calculated from the real adiponectin values in wells and is 26 ng/ml.

*The ELISA buffer is pipetted into blank wells.

> *Limit of assay*

Results exceeding adiponectin level of 100 µg/ml should be repeated with more diluted samples (e.g. 60x). Dilution factor needs to be taken into consideration in calculating the adiponectin concentration.

> *Cross-reactivity*

The antibodies used in this ELISA are specific for human adiponectin.

The assay recognizes natural and recombinant human adiponectin (full-length protein, mutation-modified trimer-only-forming protein, and globular domain).

Determination of adiponectin does not interfere with haemoglobin (1.0 mg/ml), bilirubin (170 µmol/l) and triglycerides (5.0 mmol/l).

Adiponectin was measured in some adipose tissue extracts, however, most of the extract adiponectin levels were below the assay detection limit. No cross-reactivity has been observed for human leptin, leptin receptor and resistin at 100 ng/ml.

Molecule/Species	Cross-reactivity
Bovine	No
Cat	No
Dog	No
Goat	No
Hamster	No
Horse	No

Monkey	yes
Mouse	No
Pig	No
Rabbit	No
Rat	No
Sheep	No

> *Precision*

- Intra-assay (n=8)

Sample	Mean (µg/ml)	SD (µg/ml)	CV (%)
1	11.71	0.69	5.9
2	12.28	0.481	3.9

- Inter-assay (n=8)

Sample	Mean (µg/ml)	SD (µg/ml)	CV (%)
1	8.23	0.52	6.3
2	19.86	1.39	7.0

> *Recovery test (serum samples)*

Sample	Observed (µg/mL)	Expected (µg/mL)	Recovery O/E (%)
1	5.10	-	-
	10.39	10.10	102.9
	15.57	15.10	103.1
	23.19	25.10	92.4

2	10.94	-	-
	16.18	15.94	101.5
	21.14	20.94	101.0
	30.02	30.94	100.3

> *Dilution test (serum samples)*

Sample	Dilution	Observed (µg/mL)	Expected (µg/mL)	Recovery O/E (%)
1	-	18.05	-	-
	1:2	9.28	9.02	102.8
	1:4	4.39	4.51	97.3
	1:8	2.53	2.26	112.7
2	-	23.56	-	-
	1:2	10.15	11.78	86.2
	1:4	5.64	5.89	95.8
	1:8	3.08	2.94	104.5

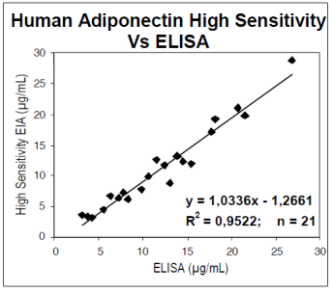
> *Effect of sample matrix*

EDTA, citrate and heparin plasmas were compared to respective serum samples obtained from healthy persons (n = 10) in the same time.

Sample	Mean (µg/mL)	Plasma /Serum (%)
Serum	8.9	-
Citrate Plasma	8.2	91.8
EDTA Plasma	8.7	97.4
Heparin Plasma	9.4	105.6

> *Method comparison*

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



Materials and equipment required

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

For the assay:

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution
- Precision micropipettes (10 to 1000 µl) with disposable tips
- Multichannel pipette (50 to 200 µl) with disposable

tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker
- Microplate washer (optional) [Manual washing is possible but not preferable]
- Microplate reader (450 nm filter +/- 10 nm, preferably with reference wavelength 630 nm, acceptable interval: 550 - 650 nm)

Sample collection and preparation

This assay can be used to measure human Adiponectin in serum and plasma (EDTA, citrate and heparin).

General precautions

- All samples must be free from organic solvents prior to assay.
- Samples should be assayed immediately after collection or should be stored at -80°C prior the use with the assay.
- Avoid using hemolyzed or lipemic samples.
- Avoid repeated freeze/thaw cycles.
- Do not store diluted samples.

Serum and plasma

Dilute samples 30x with ELISA buffer just prior to the assay, e.g. 10 µl of sample + 290 µl of ELISA buffer for duplicates.

Mix well (not to foam). Vortex is recommended.

Serum or plasma samples should be stored at -20 °C. However, no significant decline in concentration of human adiponectin was observed in serum and plasma samples after 7 days when stored at 2-8 °C.

It has been shown that the adiponectin concentration in serum or plasma samples does not decrease after repeated (5x) freeze/thaw cycles. Nevertheless, unnecessary thawing-freezing should be avoided.

Reagent preparation

All reagents need to be brought to room temperature (around +20°C) prior to the assay.

Assay reagents are supplied ready to use, except Adiponectin (human) Standards, and Concentrated Wash Buffer (10x). Stabilities after opening are summarized in the following table.

Reagent	Stability at 2-8°C after opening
Adiponectin (human) Tracer	3 months
Substrate Solution (TMB)	3 months
Stop Solution	3 months
Adiponectin (human) QC	3 months
Concentrated Wash Buffer (10x)	1 month

Please note that:

- Substrate Solution should remain colourless until

added to the plate. Keep Substrate Solution protected from light.

- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution

Quality Controls

Please note that Quality Controls are ready to use, do not dilute them. (Quality controls are supplied diluted 30x).

Refer to the vial label for current QC concentration

Stability at 2-8°C: 3 months after opening.

Adiponectin (human) Standards

Dilute each concentration of Standards 3x with the ELISA buffer just prior to the assay, e.g. 50 µl of Standard + 100 µl of ELISA buffer for duplicates. Mix well (not to foam).

Stability at 2-8°C: 3 months after opening.
Do not store the diluted Standard solutions.

Concentrated Wash Buffer (10x)

Dilute Concentrated Wash Buffer (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Concentrated Wash Buffer (10x) + 900 ml of distilled water for use in all 96 wells.

Stability at 2-8°C: 3 months for the 10x concentrated buffer and 1 month for the diluted buffer.

Assay procedure

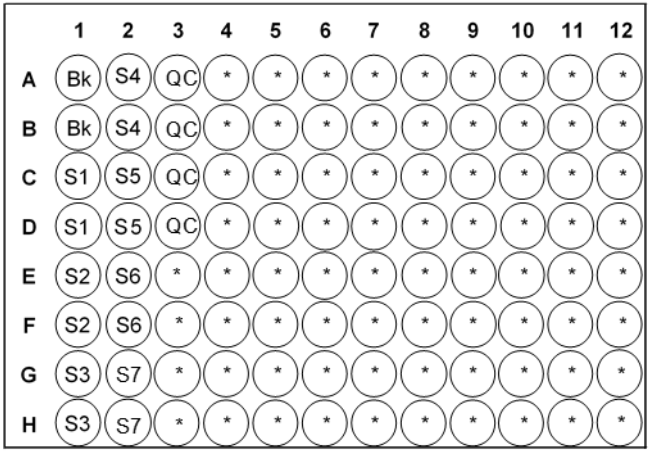
It is recommended to perform the assay in duplicate and to follow the instructions hereafter.

Plate preparation

Open the plate pouch and select sufficient strips for your assay and place the unused strips back in the pouch with the desiccant.
Stability at +4°C: 3 months.

Plate set-up

A plate set-up is suggested hereafter. The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.



Bk : Blank
QC : Quality Controls
S1-S7 : Standards 1-7
* : Samples

Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.

Use different tips to pipet the buffers, standards, samples, antibody and other reagents.

Before pipetting, equilibrate the pipette tips in each reagent. Do not touch the liquid already in the well when expelling with the pipette tip.

> ***ELISA Buffer***

Dispense 50 µL to the blank (Bk) wells.

> ***Adiponectin (human) Standards***

Dispense 50 µL of each Standard (S7 to S1) in duplicate into the appropriate wells.

Start with the lowest concentration standard (S7) and equilibrate the tip in the next higher standard before pipetting.

> ***Adiponectin (human) Quality Controls***

Dispense 50 µL of Quality Controls in duplicate into appropriate wells.

> ***Samples***

Dispense 50 µL in duplicate into appropriate wells.

> ***Adiponectin (human) Tracer***

Dispense 50 µL to each well, except the blank (B) wells.

▷ Incubating the plate

Cover the plate with the cover sheet and incubate for 2 hours at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Developing and reading the plate

- Rinse each well 3 times with 350 µL of Wash Buffer. Slightly shake the plate for 5 minutes (with orbital shaker).
- At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.
- Add 200µL of Substrate Solution to each well.
- Incubate in the dark during 10-15 minutes 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.
- Add 50µL of Stop Solution to each well.
- Read the absorbance at 450 nm within 5 minutes following stop solution addition. Preferably set up the reference wavelength at 630 nm (acceptable range: 550 - 650 nm).

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.

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)			
	Blank	Standards	Sample or QC
Standards	-	50	-
Sample or QC	-	-	50
Tracer	-	50	50
Cover plate, incubate **2 hours** at room temperature under orbital shaking at 300 rpm			
Wash wells 3 times with 350 µL/well Discard liquid from the wells & dry on absorbent paper			
Substrate 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

- Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm.
- Subtract the absorbance readings of the blank wells from the absorbance readings of the rest of the plate.
- Calculate the average absorbance for each standard, QC and sample.
- For each standard, plot the absorbance on y axis versus the concentration on x axis. 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 samples.
- Most plate readers are supplied with a curve-fitting software capable of graphing these data (logit/log or 4-

parameter logistic fit 4PL). If you have this type of software, we recommend using it. Refer to it for further information.

- Since Standards are diluted at 1:3 while Quality Controls and Samples have been diluted at 1:30, the measured concentration of Samples and Quality controls calculated from the standard curve must be multiplied by a factor of 10.

Acceptable range

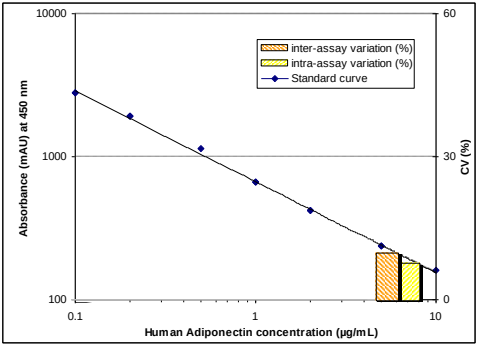
- QC samples: see label on the vials.

Typical results

The following data are for demonstration purpose only. Your data may be different and still 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)	m A.U.
Standard 10 µg/ml (S1)	160
Standard 5 µg/ml (S2)	236
Standard 2 µg/ml (S3)	418
Standard 1 µg/ml (S4)	661
Standard 0.5 µg/ml (S5)	1 136
Standard 0.2 µg/ml (S6)	1 935
Standard 0.1 µg/ml (S7)	2 788
QC High	381
QC Low	807

HUMAN ADIPONECTIN STANDARD CURVE



Troubleshooting

Absorbance values are too low:

- one reagent has not been dispensed,
- incorrect preparation,
- assay performed before reagents reached 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,
- irregular plate washing.

These are a few examples of troubleshooting that may occur. If you need further explanation, Bertin Bioreagent will be happy to assist you. Feel free to contact our Technical Support staff by phone +33 (0)139 306 036, fax +33 (0)139 306 299 or E-mail tech@bertin-bioreagent.com, and be sure to indicate the batch number of the kit (see outside the box).

Bertin Bioreagent proposes ELISA Training kit #B05005 and EIA workshop upon request. For further information, please contact our Technical Support.

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	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

With 30 years of experience, Bertin Bioreagent develops and sells best-in-class kits and products for life science research labs. Our scientist team innovate each day to tailor biomarker assays, pre-analytical products, kits, antibodies and biochemicals that are ready to use, fully validated with a strict quality control.
We strive to address a broad range of research interest: inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, pain, prion diseases.

Bertin Bioreagent has also a long expertise in developing customized solutions adapted to your need. Feel free to contact us for your special projects!

To offer a complete solution to researchers, Bertin Instruments offers a range of unique laboratory equipment from Air Sample collection, Sample Homogenisation and Digital Imaging.

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