Adiponectin High Sensitivity (human)

For laboratory research only. Not for human or veterinary diagnostic use.

Bertin Bioreagent also markets pre-analytical products, ELISA kits, antibodies & biochemicals for:

- **Inflammation**
- **Oxidative injury**
- **Endocrinology**
- **Diabetes**
- **Obesity**
- **Hypertension**
- **Pain**
- **Prion diseases**

Adiponectin High Sensitivity (human)
ELISA kit
#A05186.96 wells

For research laboratory use only
Not for human diagnostic use

Fabriqué en France
Made in France

#A11186
Version: 0122

Table of contents

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

This kit contains:

Designation	Item #	Quantity per kit	Form
Adiponectin High Sensitivity (human) precoated Microtiter plate	A08186.1 ea	1	-
Adiponectin High Sensitivity (human) Tracer	A40186.100 dtn	1	Liquid
Adiponectin High Sensitivity (human) Standard (1, 2, 5, 10, 20, 50, 100 and 150 ng/mL)	A06186_X.1 ea	8x1	Liquid
Adiponectin High Sensitivity (human) QC High	A10186_H.1ea	1	Liquid
Adiponectin High Sensitivity (human) QC Low	A10186_L.1ea	1	Liquid
ELISA Buffer 10x	A07012.1 ea	1	Liquid
Concentrated Wash Buffer 10x	A17012.1 ea	1	Liquid
Substrate Solution (TMB)	A09010.100 dtn	1	Liquid
Stop Solution	A22000.100 dtn	1	Liquid
Well cover sheet	-	1	-
Technical Booklet	A11186.1 ea	1	-

Each kit contains sufficient reagents for 96 wells. This allows the assay of 40 samples in duplicate.

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 in which kit reagents are handled
- Do not mix different lot numbers
- 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 potential harmful solution. To avoid any contact, wear eye, hand, face and

clothing protection when handling these reagents. Wearing gloves, laboratory coat and glasses is recommended when assaying kit materials and samples.

Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), that 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 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.

Principle of the assay

This Enzyme Immunometric Assay (EIA/ELISA) is based on a sandwich technique. The wells of the plate supplied are coated with a polyclonal antibody specific to human adiponectin.

Adiponectin introduced into the wells (standard or sample) will be bound by the polyclonal antibody coated on the plate and is then detected by a second polyclonal antibody tagged with horseradish peroxidase (HRP) also specific for adiponectin.

The two antibodies then form a sandwich by binding on different parts of the human adiponectin.

The sandwich is immobilised on the plate so reagents in excess may be washed away. The concentration of adiponectin is determined by measuring the enzymatic activity of immobilized Tracer using TMB. The Tracer acts on TMB to form a yellow compound after the reaction has been stopped.

The intensity of the colour, which is determined by spectrophotometry at 450 nm, is proportional to the amount of adiponectin present in the well during the immunological

incubation.

Assay characteristics

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

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: Ablank + 3*SD_{blank}) is:

- 0.47 ng/mL for plasma and serum samples,
- 0.156 ng/mL for urine and CSF samples.

*The EIA buffer was pipetted into blank wells.

> *Cross-reactivity*

The assay recognizes human and recombinant adiponectin.

Molecule/Species	Cross-reactivity
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	yes

> *Precision*

- Intra-assay (n=8)

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	6.34	0.28	4.4
2	9.41	0.31	3.3

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

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	9.41	0.54	5.8
2	17.74	1.11	6.2

> *Recovery test (serum samples)*

Sample	Observed (µg/mL)	Expected (µg/mL)	Recovery O/E (%)
1	4.65	-	-
	21.90	24.34	90.0
	14.40	15.57	92.5
	10.16	10.10	100.6
2	7.79	-	-
	23.87	27.48	86.9
	15.58	18.71	83.3
	11.87	13.24	89.7

> *Dilution test (serum samples)*

Sample	Dilution	Observed (µg/mL)	Expected (µg/mL)	Recovery O/E (%)
1	-	14.21	-	-
	1:2	6.51	7.11	91.6
	1:4	4.05	3.55	113.9
	1:8	1.73	1.78	97.3
2	-	19.98	-	-
	1:2	10.51	9.99	105.2
	1:4	5.40	5.00	108.1
	1:8	2.35	2.50	94.1

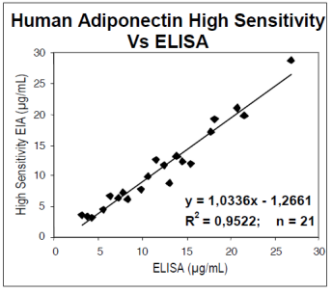
> *Serum / Plasma Samples*

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

Sample	Mean (µg/mL)	Plasma /Serum (%)
Serum	12.4	-
Citrate Plasma	10.3	83.6
EDTA Plasma	12.0	96.8
Heparin Plasma	12.3	99.6

> *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:

- Precision micropipettes (5 to 1000 µL)
- Spectrophotometer plate reader (450 nm filter +/- 10 nm, preferably with reference wavelength 630 nm, acceptable interval: 550 - 650 nm)
- Microplate washer (or washbottles)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 100 µL
- Distilled or deionised water
- Polypropylene tubes

Sample collection and preparation

This assay can be used to measure human Adiponectin in human samples such as serum, plasma, breast milk, urine, CSF.

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

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 at 1:300 in ELISA buffer preferably in two steps:

- add 10 µL of sample to 90 µL of ELISA buffer (10x dilution). Mix well.
- Add 10 µL of this solution to 290 µL ELISA buffer (30x dilution) for the final dilution. Mix well.

Serum or plasma samples should be stored frozen (preferably at -80 °C, then the stability is at least 1 year). It has been shown that the adiponectin concentration in serum or plasma samples does not decrease after five thawing-freezing cycles. Nevertheless, repeated thawing-freezing should be avoided.

Breast milk, CSF and urine samples

Dilute samples at 1:3 in ELISA buffer (i.e. 100 µL sample + 200 µL ELISA buffer), just prior to the assay. Mix well, without foaming.

Stability of milk, CSF and urine samples have not been tested.

Reagent preparation

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

Centrifuge the liquid contained in microtube vials before opening.

Assay reagents are supplied ready to use, except Quality Controls, ELISA buffer and wash buffer. Stabilities after opening are summarized in the following table.

Reagent	Stability at 2-8°C after opening
Adiponectin High Sensitivity (human) Tracer	3 months
Substrate Solution (TMB)	3 months
Stop Solution	3 months
Adiponectin High Sensitivity (human) Standards	3 months

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

ELISA Buffer

Dilute only required amount of concentrated ELISA buffer (10x). Otherwise dilute all 20 ml of concentrated ELISA buffer (10x) with 180 ml of distilled or deionised water to prepare 200 ml of ELISA Buffer (1x) for use of all-wells.
Stability at 2-8°C: 3 months for the 10x concentrated buffer and 1 week for the diluted buffer.

Quality Controls

Please note that Quality Controls are supplied diluted 30x.
Dilute Quality Control (HIGH and LOW) 10x with the ELISA Buffer (1x) just prior to the assay. For example: 30 µl of QC + 270 µl of ELISA Buffer for duplicates. Mix well (not to foam). Vortex is recommended. Beware of imprecision in pipetting.
Stability at 2-8°C: 3 months after opening. Do not store the diluted quality controls.

Wash Buffer

Dilute Concentrated Wash Buffer (10x) ten-fold in distilled or deionised water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.*Stability at 2-8°C: 3 months for the 10x concentrated buffer and 1 month for the diluted buffer.*

Assay procedure

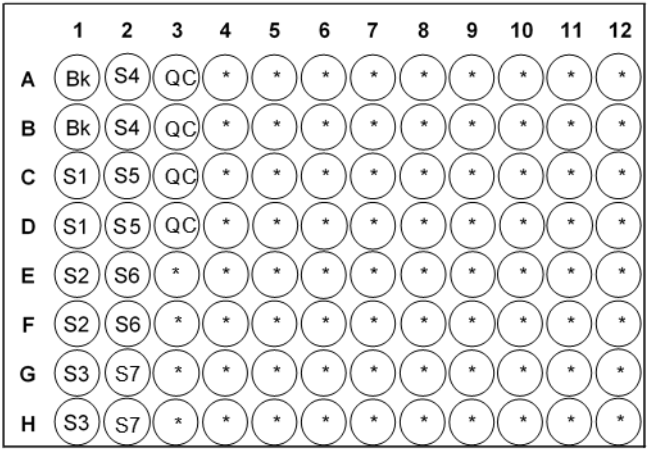
It is recommended to perform the assays in duplicate following the instructions hereafter.

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 100 µL to the blank (Bk) wells.

Adiponectin High Sensitivity (human) Standards

Dispense 100 µL of each standard (5 to 150 ng/mL for serum or plasma samples and 1 to 50 ng/mL for milk or urine or CSF samples) in duplicate to the appropriate wells.

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

Adiponectin Quality Controls and Sample

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

Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Washing the plate

Rinse each well 3 times with Wash Buffer (350 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents

- ***Adiponectin High Sensitivity (human) Tracer***

Dispense 100 µL to each well. Mix gently.

Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes 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. 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 100µL of Substrate Solution to each well.

- Incubate in the dark during 10 minutes for serum or plasma samples or 30 minutes for milk or urine or CSF 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.

- Add 100µL 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	Standard	Sample or QC
ELISA buffer	100		
Standard	-	100	-
Sample or QC	-	-	100
Cover plate, incubate **60** minutes 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			
Tracer	100	100	100
Cover plate, incubate **60** minutes 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	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 samples.			
Stop Solution	100	100	100
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 (4-parameter logistic fit 4PL). If you have this type of software, we recommend using it. Refer to it for further information.
- 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.

Acceptable range

- QC samples: see label on the vials or see quality control sheet

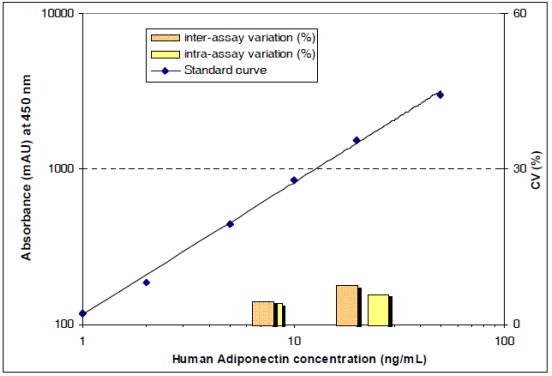
Typical results

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.
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)	m A.U.
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

Typical 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.

Bibliography

Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem. 1995, 270, 26746-26749.*

Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (adipose most abundant gene transcript 1). *Biochem Biophys Res Commun. 1996, 221, 286-289.*

Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun. 1999, 257, 79-83.*

Saito K, Tobe T, Minoshima S, Asakawa S, Sumiya J, Yoda M, Nakano Y, Shimizu N, Tomita M. Organization of the gene for gelatin-binding protein (GBP28). *Gene. 1999, 229, 67-73.*

Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of

macrophages. *Blood. 2000, 96, 1723-1732.*

Das K, Lin Y, Widen E, Zhang Y, Scherer PE. Chromosomal localization, expression pattern, and promoter analysis of the mouse gene encoding adipocyte-specific secretory protein Acrp30. *Biochem Biophys Res Commun. 2001, 280, 1120-1129.*

Tsao TS, Lodish HF, Fruebis J. ACRP30, a new hormone controlling fat and glucose metabolism. *Eur J Pharmacol. 2002, 440, 213-21.*

Wang Y, Xu A, Knight C, Xu LY, Cooper GJ. Hydroxylation and glycosylation of the four conserved lysine residues in the collagenous domain of adiponectin. Potential role in the modulation of its insulin- sensitizing activity. *J Biol Chem. 2002, 277, 19521-19529.*

Kondo H, Shimomura I, Matsukawa Y, Kumada M, Takahashi M, Matsuda M, Ouchi N, Kihara S, Kawamoto T, Sumitsuji S, Funahashi T, Matsuzawa Y. Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. *Diabetes. 2002, 51, 2325-2328.*

Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Adiponectin and protection against type 2 diabetes mellitus. *Lancet. 2003, 361, 226-228.*

Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE. Structure-function studies of the adipocyte-secreted

hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. *J Biol Chem. 2003, 278, 9073-9085.*

Daimon M, Oizumi T, Saitoh T, Kameda W, Hirata A, Yamaguchi H, Ohnuma H, Igarashi M, Tominaga M, Kato T. Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese Population: the Funagata study. *Diabetes Care. 2003 Jul;26(7):2015-20.*

Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, Uchida S, Ito Y, Takakuwa K, Matsui J, Takata M, Eto K, Terauchi Y, Komeda K, Tsunoda M, Murakami K, Ohnishi Y, Naitoh T, Yamamura K, Ueyama Y, Froguel P, Kimura S, Nagai R, Kadowaki T. Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem. 2003, 278, 2461- 2468.*

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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.

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