invitron
high sensitivity diagnostics

IV3-102E

English

Adiponectin (total) ELISA Kit

For in-vitro diagnostic use only

CE

Invitron Ltd.
Wyastone Business Park, Wyastone Leys
Monmouth NP25 3SR, UK
www.invitron.com

Definitions

 Instructions for use

REF Catalogue number

 Use by

LOT Lot/Batch Code

 Storage temperature limitations

IVD In vitro diagnostic medical device

Contains sufficient for <N> tests

Invitron Adiponectin (total) Kit

Intended Use

The Invitron Adiponectin kit is an ELISA for the quantitative measurement of adiponectin in human serum and plasma samples. It is for *in vitro* diagnostic use only.

Summary and Explanation

Adiponectin is a protein of 244 amino acids and a molecular weight of approximately 30 kDa. Its concentration in blood is 5-30 µg/ml. Adiponectin has four structural domains: a signal peptide, a variable domain, a collagen-like N-terminal domain and a globular C-terminal domain. It exists *in vivo* in different oligomeric forms. It occurs as a trimer and a ditrimer as well as high molecular weight multimers. It is expressed mainly by adipocytes, though it is also synthesised by hepatocytes and muscle cells. To date, the only known natural inducer of adiponectin synthesis is IGF-1. Two different adiponectin receptors are known: Adipor1 is synthesised in muscle and Adipor2 in liver tissue.

The role of adiponectin in the human is not fully understood. Studies have shown that circulating adiponectin correlates negatively with BMI and may affect energy metabolism through the regulation of fatty acid oxidation. Adiponectin may be involved in physiological processes such as angiogenesis as well as in glucose and lipid metabolism. Circulating levels are reduced in type 2 diabetes. Adiponectin may modulate the inflammatory process and may therefore be of significance in the development of arteriosclerosis and coronary heart disease. Blüher *et al* (2007) showed a correlation between total adiponectin and insulin sensitivity and demonstrated the ability of total adiponectin to predict insulin resistance and impaired glucose tolerance. Low circulating adiponectin may result in the inhibition of fatty acid oxidation and may act as a marker for insulin resistance and arteriosclerosis.

Indications

- Energy metabolism and body weight regulation
- Type 2 diabetes
- Coronary artery disease
- Atherosclerosis

Principle

The Invitron Adiponectin ELISA is a two-site assay for the determination of total adiponectin in serum or plasma samples. The wells of the microtitre plate are coated with a monoclonal antibody raised against human adiponectin. Samples and standards are incubated in the wells in order to capture adiponectin. After washing to remove all unbound material a peroxidise-labelled polyclonal antibody is added and a further incubation is carried out. After another wash step, the peroxidise substrate tetramethylbenzidine (TMB) is added and the colour allowed to develop. The reaction is terminated by addition of acid and the absorbance read at a wavelength of 450 nm. Absorbance is proportional to the concentration of adiponectin in the sample and results are calculated by reference to a calibration curve generated from standard concentrations.

Materials Provided

- Coated Microtitre Plate (a)
- (12 x 8) stripwells coated with a monoclonal antibody to adiponectin. The plate is sealed inside a foil pouch with a desiccant to maintain a moisture-free environment.
- Labelled Antibody (b)
- (1 x 150 µl) peroxidise-labelled anti-adiponectin antibody

- Sample Diluent (c)
- (2 x 100 ml) Buffer for dilution of samples, ready to use
- Standards (d-i)
- (2 x 5 vials; 1 ml) Calibrators 0; 1.4; 5.5; 22; 88 ng/ml, lyophilised
- Controls (j, k)
- (2 x high and low; 1 ml) low and high controls, lyophilised
- Substrate (l)
- Tertramethylbenzidine (TMB) substrate, ready to use
- Stop Solution (m)
- Dilute sulphuric acid, ready to use
- Wash Buffer Concentrate (n)
- (10x concentration) Phosphate buffered saline containing detergent and preservative

Materials Required but not Supplied

- Deionised water
- Precision pipettes and tips able to deliver 10-1000 µl
- Plate sealers
- Vortex mixer
- Microtitre plate shaker
- Microtitre plate reader

Warnings and Precautions

- Human materials used in the kit components have tested negative for HIV, hepatitis B and hepatitis C. Nevertheless, for safety reasons, all components should be treated as potentially infective.
- Stop solution is diluted sulphuric acid and therefore must be handled with care. It can cause burns and so gloves, safety glasses and appropriate protective clothing should be worn when handling it. Any spills should be wiped up immediately using large volumes of water.
- Kit reagents contain thiomersal which is toxic. The substrate for the enzyme (TMB) is carcinogenic. Care should be taken to avoid any skin contact with these reagents.
- Reagents should not be used beyond the expiry date shown on the kit label.

Preparation and Storage of Reagents

- Up to 4 runs can be carried out with each kit. **Prepare only the required quantity of reagents for each run.** Ensure that the reagents are stored under the conditions stated on the kit label.
- Wash buffer concentrate should be diluted 1:10 in deionised water before use. If any crystals have formed during storage, these should be re-dissolved by warming the wash buffer to 37º prior to dilution. Wash buffer concentrate is stable when stored at 2-8º up to the expiry date on the label. Diluted wash buffer can be stored in a closed vessel for up to 4 weeks at 2-8º.
- Lyphilised standards and controls are stable when stored at 2-8º until the expiry date stated on the label.
- All other test reagents are ready to use. These reagents are stable until the expiry date given on the kit label when stored at 2-8º.

Specimen Collection and Preparation

Serum: Whole blood should be taken into a plain tube and allowed to clot for 30 minutes. The clot should be separated by centrifugation. Care should be taken to avoid haemolysis.

Plasma: Whole blood should be collected into a tube containing EDTA anticoagulant and centrifuged immediately after collection.

Before performing the assay, it is necessary to dilute serum or plasma 1:1000 with Sample Diluent. This factor must then be taken into account when calculating results from the calibration curve. It is recommended that dilution be carried out in two steps as follows:

1. Add 25µl sample to 975µl of sample diluent (1:40)
2. Add 40µl of dilution 1 to 960µl of sample diluent (1:25)

For analysis, add 100µl of dilution 2 to the respective well.

Samples may be stored for up to 1 year prior to analysis and will not be affected by up to 3 freeze/thaw cycles.

Assay Procedure

1. Bring all kit components and samples to room temperature before use and mix well.
2. Produce a protocol sheet recording the position of standards, controls and samples.
3. Assemble the required number of coated strips in the plate holder. Any strips not used immediately may be stored inside the foil pouch with silica gel desiccant. Make sure to fill remaining spaces in the plate holder with uncoated strips to ensure uniform heat transfer during incubation.
4. Wash each well 5 times with a volume of 250 µl of diluted wash buffer. After the final wash remove residual buffer by tapping gently on absorbent paper.
5. Add 100 µl of standard, control and sample to the respective wells in duplicate.
6. Cover the plate with a plate sealer and incubate for 1 hour at room temperature on a plate shaker.
7. Discard the contents of the wells. Wash 5 times with a volume of 250 µl of diluted wash buffer. After the final wash remove residual buffer by tapping gently on absorbent paper.
8. Add 100 µl of labelled antibody into each well.
9. Cover the plate with the sealer and incubate for 1 hour at room temperature on a plate shaker.
10. Discard the contents of the wells. Wash 5 times with a volume of 250 µl of diluted wash buffer. After the final wash remove residual buffer by tapping gently on absorbent paper.
11. Add 100 µl of substrate into each well.
12. Incubate for 10-20 minutes at room temperature **in the dark.***
13. Add 50 µl of stop solution into each well.
14. Read the absorption in an ELISA reader at a wavelength of 450 nm.

*The rate of colour change is temperature dependent. It is recommended that the development reaction is stopped when the colour in the standards shows good discrimination.

Results

The results may be calculated automatically using a cubic spline or 4-parameter curve fit. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard should be further diluted**. For the calculation of the concentrations in serum/plasma or urine samples, the appropriate dilution factor must be taken into account.**

Limitations

- Only if test instructions are rigidly followed will optimum results be achieved.
- Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as thorough mixing of all prepared solutions.
- While rinsing, check that all wells are filled evenly with wash buffer, and that there are no residues in the wells.

Quality Control

The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs where possible in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. If, after checking the above mentioned items, there is no error, contact Invitron directly.

Expected Values

Based on a study of 80 healthy individuals, a mean adiponectin concentration of 8.8 µg/ml was found in plasma. Plasma adiponectin levels are reduced in patients with impaired glucose tolerance.

It is recommended that each laboratory establishes its own expected ranges for its patient population.

Performance

Precision

Inter-assay precision of the Invitron Adiponectin ELISA was calculated from the results obtained for 12 replicate determinations at two dose levels.

Sample	Mean Adiponectin (µg/ml)	CV (%)
1	6.9	6.3
2	8.4	6.1

Recovery

Two samples were spiked with three different amounts of adiponectin and then assayed in the Invitron Adiponectin ELISA. The following table compares the measured results with those expected from the spiked samples.

Sample	Spike (ng/ml)	CRP Expected (ng/ml)	CRP Measured (ng/ml)
A (3.8 ng/ml)	2.0	5.8	6.1
	4.0	7.8	7.6
	6.0	9.8	9.2
B (2.1 ng/ml)	5.0	7.1	8.1
	7.0	9.1	9.6
	9.0	11.1	10.8

Linearity

Two patient samples were diluted and measured in the Invitron Adiponectin ELISA. The results are shown in the following table.

Sample	Dilution	CRP Expected (µg/ml)	CRP Measured (µg/ml)	Recovery (%)
A	1:600	7.65	7.65	100
	1:1200	3.83	4.14	107
	1:2400	1.91	2.33	122
	1:4800	0.96	1.23	128
B	1:300	7.60	7.60	100
	1:600	3.80	3.87	102
	1:1200	1.90	2.11	111
	1:2400	0.95	1.17	123

Reference

Blüher M *et al*. (2007) Total and high-molecular weight adiponectin in relation to metabolic variables at baseline and in response to an exercise treatment program: comparative evaluation of three assays. Diabetes Care; 30: 280-285.

For additional information and product support please contact:
Invitron Limited
Wyastone Business Park, Wyastone Leys, Monmouth NP25 3SR, UK
Tel: +44 (0)1600 891536
Fax: +44 (0)1600 891537
info@invitron.com
www.invitron.com