Manual

Adiponectin (total) ELISA

For the in vitro determination of human adiponectin in serum and plasma

Valid from 2020-02-04

REF | **K 6250**

Σ
96

+8°C
+2°C

IVD

CE

Table of Contents

1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of adiponectin in serum and plasma. For *in vitro* diagnostic use only.

2. INTRODUCTION

Adiponectin consists of 244 amino acids and has an approximate molecular weight of 30 kDa. The adiponectin concentration in blood is 5–30 µg/ml that accounts for about 0.01 % of the total plasma proteins. Adiponectin has four domains: a signal peptide, a variable domain, a collagen-like N-terminal domain and a globular C-terminal domain. Adiponectin exists in different oligomer forms *in vivo*. Beside the trimer and ditrimer also high molecular multimers are present. Adiponectin is mainly expressed by adipocytes, but also muscle cells and hepatocytes can synthesise it. One of the natural inductors of the adiponectin synthesis is IGF-I. Two different adiponectin receptors are known. Both of them are ubiquitary expressed, though their distribution in the tissues varies: Adiponectin receptor 1 (AdipoR1) is synthesised in muscle and AdipoR2 in liver tissue. The adiponectin significance for the human organism is not completely clear until now. Studies show, that adiponectin correlates negatively with BMI and could participate at the energy metabolism through the regulation of fatty acid oxidation. Adiponectin influences further physiological processes like angiogenesis. It is also associated with glucose and lipid metabolism. Adiponectin levels are associated with insulin resistance and accordingly linked with type 2 diabetes. Furthermore, it is involved in inflammatory processes and therewith of importance for development of arteriosclerosis and coronary artery diseases. Blüher et al. (2007) showed a correlation of total serum adiponectin to insulin sensitivity and the ability of total serum adiponectin levels to predict insulin resistance and impaired glucose tolerance. Low adiponectin concentrations result in inhibition of fatty acid oxidation and are associated with insulin resistance and metabolic syndrome as well as arteriosclerosis.

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

3.   MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6250	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 6250	WASHBUF	Wash buffer concentrate 10x	2 x 100 ml
K 0001.C.100	SAMPLEBUF	Sample dilution buffer	2 x 100 ml
K 6250	STD	Adiponectin standards, lyophilised (0; 1.4; 5.5; 22; 88 ng/ml)	2 x 5 vials
K 6250	CTRL1	Control, lyophilised (see specification for range)	2 x 1 vial
K 6250	CTRL2	Control, lyophilised (see specification for range)	2 x 1 vial
K 6250	CONJ	Conjugate, ready-to-use	1 x 15 ml
K 0002.15	SUB	Substrate (Tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as purchase order number.

4.   MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

  * Immundiagnostik AG recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.

- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultra pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.

- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification data sheet**. **Standards and controls** (reconstituted STD and CTRL) **are stable at –20 °C for 4 days and can be frozen and thawed for up to 2 times.**

- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Serum and plasma

Storage of samples

Samples can be stored for **two years at -20 °C** and up to **two times frozen and thawed**. The samples are stable at **room temperature or 2–8 °C** for **up to 7 days**.

Preparation of samples

EDTA plasma or serum samples must be diluted **1:1 000** before performing the assay. For example:

- **25 µl** sample + **975 µl** sample dilution buffer, mix well = **1:40** (**dilution I**)
- **40 µl** dilution I + **960 µl** sample dilution buffer, mix well = **1:25** (**dilution II**). This results in a **final dilution of 1:1 000**.

For analysis, pipette **100 µl** of dilution II per well.

7. ASSAY PROCEDURE

Principle of the test

The assay utilises the two-site sandwich technique with one monoclonal and one polyclonal antibody that bind to human adiponectin.

Standards, controls and diluted patient samples which are assayed for human adiponectin are added to wells of a microplate coated with a high affinity monoclonal anti-human adiponectin antibody. During the first incubation step, adiponectin in the samples is bound by the immobilised antibody. Then a peroxidase labelled conjugate is added to each well and the following complex is formed: capture antibody - human adiponectin – peroxidase conjugate. Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The colour changes from blue to yellow. The intensity of the yellow colour is directly proportional to the adiponectin concentration. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Adiponectin, present in the patient samples, is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.

4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add **100 µl conjugate** (CONJ) into each well.
6.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) into each well and mix well.
11.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3.   **Spline algorithm**

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum and plasma samples

The obtained results have to be multiplied by the **dilution factor of 1 000** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

9.   LIMITATIONS

Samples with concentrations above the measurement range can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used

LoB see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

A significant correlation between adiponectin serum values and age as well as gender of the probands is reported, in turn the correlation between the respective BMI seems to be less significant.

Serum and Plasma (n = 80): 8.79 µg/ml

Based on Immundiagnostik AG studies of apparently healthy individuals (n = 80) a mean value of 8.79 µg/ml was determined.

Company	Adiponectin mean value* [µg/mL]		
Adiponectin total - Kit	Normal glucose tolerance	Impaired glucose tolerance	Type 2 diabetes
LINCO	8.95 ± 0.55	3.38 ± 0.26	3.48 ± 0.42
Mediagnost	8.81 ± 3.43	3.51 ± 1.47	3.82 ± 2.16

*Values from Reference 1.

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 40

The repeatability was assessed with 2 serum samples under **constant** parameters (same operator, instrument, day and kit lot).

Sample	Mean value [µg/ml]	CV [%]
1	13.71	3.9
2	9.33	2.8

Reproducibility (Inter-Assay); n = 37

The reproducibility was assessed with 2 serum samples under **varying** parameters (different operators, instruments, days and kit lots).

Sample	Mean value [µg/ml]	CV [%]
1	12.83	5.9
2	15.25	6.4

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, adiponectin spikes with known concentrations were added to 2 different serum samples. The results below were obtained without consideration of the sample dilution factor.

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
6.53	3.7	10.20	10.51	103.07
	5.5	12.03	11.04	91.71
	7.3	13.87	13.41	96.67
	11.0	17.53	16.51	94.14
6.37	5.5	11.87	10.78	90.87
	9.2	15.53	14.40	92.72
	12.8	19.20	17.40	90.61
	16.5	22.87	19.84	86.74

Analytical sensitivity

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB   0.493 ng/ml

Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to adiponectin. There was no cross-reactivity observed.

Substance tested	Concentration added	Concentration obtained [ng/ml]	Conclusion
Resistin	10 µg/ml	< 0.493	< LoB
h Leptin	100 ng/ml	< 0.493	< LoB
h-MDA ApoB-100	1 mg/ml	< 0.493	< LoB
MDA-LDL	1 mg/ml	< 0.493	< LoB

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline EP06-A with a serial dilution of 2 different serum samples.

For adiponectin in serum and plasma, the method has been demonstrated to be linear from 3.48 to 56.36 ng/ml based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than ±20 % in this interval.

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
A	1:300	55.70	55.70	100.00
	1:600	27.85	24.09	86.50
	1:1200	13.93	12.02	86.28
	1:2400	6.96	6.64	95.29
	1:4800	3.48	3.81	109.38
B	1:300	56.36	56.36	100.00
	1:600	28.18	24.44	86.74
	1:1200	14.09	12.25	86.95
	1:2400	7.04	6.69	94.89
	1:4800	3.52	3.74	106.09

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.

- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.

- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.

- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.

- Control samples should be analysed with each run.

- Reagents should not be used beyond the expiration date stated on kit label.

- Substrate solution should remain colourless until use.

- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

- Avoid foaming when mixing reagents.

- Do not mix plugs and caps from different reagents.

- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.

- The guidelines for medical laboratories should be followed.

- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

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Used symbols:

(thermometer symbol)	Temperature limitation	REF	Catalogue Number
IVD	In Vitro Diagnostic Medical Device	→REF	To be used with
(manufacturer symbol)	Manufacturer	∇Σ	Contains sufficient for \<n> tests
LOT	Lot number	(hourglass symbol)	Use by
⚠	Attention	i	Consult instructions for use
S	Consult specification data sheet		