

Adiponectin - ELISA

(human Adiponectin, Arcp30, AdipoQ)

**Enzyme Immunoassay for Quantitative Determination
of
human Adiponectin**

Product-Code: E09
(96 Determinations)



DE/CA40/00809/18

For In-Vitro Use Only!
In the USA: For Research Use Only!



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CLINICAL IMPLICATION

- Adiposity
- Arteriosclerosis
- Energy metabolism
- Coronary diseases

TECHNICAL FEATURES+APPLICATIONS

The Mediagnost **Adiponectin ELISA E09** –

- is suited for Adiponectin determination in **Serum** and **Plasma** samples, expectation values were determined
- is extremely **sensitive** (less than 0.6 ng/ml) and, thus allows measurements in cell culture media too and in specimens others than serum or plasma
- is **fast**: incubation time a total of 1 hour and 45 minutes
- Single Standards with **1, 10, 30, 70, 100 ng/ml** human Adiponectin are provided in the Kit, Control Serum KS is of human serum
- is calibrated with native **Adiponectin** and correlated to a commercialised radioimmunoassay
- uses **high affinity monoclonal antibodies** against human Adiponectin
- Microtiter plates are separately breakapart, tests can be adapted to individual requirements

INTRODUCTION

Adiponectin is a 30kDa protein which percentage in serum proteins is 0.01%. It is mainly synthesized by Adipocytes, but also muscle cells and hepatocytes have the ability to synthesize Adiponectin. Until now, IGF-I is the only known natural inductor of the synthesis. It consists of a Collagen-like N-terminal and a globular C-terminal domain (1). In vivo Adiponectin appears with different oligomers. Beside the trimer and dimer also high molecular multimers exist (1-3). Up to now two different receptors are known, both receptors are ubiquitarily expressed, though the distribution in the tissues varies. The Adiponectin Receptor 1 (AdipoR1) is especially in muscle- and AdipoR2 in liver tissue synthesized (4).

The significance for the human organism is not clear until now. First studies show, that adiponectin correlates negatively with BMI and thus it could have relevance for the energy metabolism for example through the regulation of fatty acid oxidation. Beside the correlation with BMI, Adiponectin level is associated with the Insulin-Resistance (5-7) and so also linked with Type II Diabetes. Adiponectin is associated also with glucose- und lipometabolism (8, 9).

Furthermore it is involved in inflammatory processes (10-14) and therewith it is of importance for appearance of arteriosclerosis (4, 5, 15) and coronaritis (16, 17), thus the determination of Adiponectin level in plasma could serve to estimate the risk of coronary disease (18, 19). Beside this Adiponectin influences further physiological processes as for example the angiogenesis (20, 21).

METHODOLOGY

Assay Characteristic and Validation

The Mediagnost ELISA for Adiponectin E09 is a so-called Sandwich-Assay using two specific and high affinity antibodies. The Adiponectin

in the samples binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-Adiponectin-Antibody binds in turn to the immobilised Adiponectin. The second antibody is biotinylated and will be applied in a mixture with a Streptavidin-Peroxidase-Enzyme Conjugate. In the closing substrate reaction the turn of the colour will be catalysed quantitatively depending on the Adiponectin-level of the samples.

The Standards of the ELISA E09 are prepared from native Adiponectin (Human Serum) in concentrations of 1, 10, 30, 70 and 100 ng/ml. The native Adiponectin was quantified with a recombinant protein and with a commercialised radio immunoassay (Linco Corp.) for Adiponectin.

The **recovery** of recombinant Adiponectin yielded in a serum matrix on average 100%.

The **analytical sensitivity** of the ELISA E09 yields < **0.6 ng/ml** (equal to < **0.06** ng per well; as 2xSD of zero standard in 16fold determination).

The Mediagnost Adiponectin ELISA E09 is over a very wide range dilution authentic, the linearity of serum dilutions is over a very wide range excellent (s.Tab.1).

Table 1: The linearity of the sample dilution
(characteristic results of two different sera)

Dilution:	Sample 1 (recalculated, µg/ml)	Dilution:	Sample 2 (recalculated, µg/ml)
1:200	12.49	1:200	11.58
1:400	11.92	1:400	11.74
1:600	10.80	1:600	11.41
1:800	11.17	1:800	11.35
1:1000	12.06	1:100	10.58
1:1200	11.64	1:1200	10.96
1:1400	10.86	1:1400	11.18
1:1600	10.75	1:1600	10.61
AV / 1SD / VC%	11.46 / 0.66 / 5.8	AV / 1SD / VC%	11.18/ 0.43 / 3.8

AV= Average Value, SD = Standard deviation , VC = Variation coefficient

The **Inter-** and **Intra-Assay** Variations coefficients were found **less than 6.7%** and **4.7% respectively**. Exemplary determinations are shown in table 2 and table 3.

Table 2 : Inter-Assay-Variation

	Number of single determinations	Mean value (µg/ml)	Standard deviation (µg/ml)	VC (%)
Sample 1	22	4.76	0.28	5.88
Sample 2	25	5.22	0.35	6.72
Sample 3	25	5.62	0.32	5.70
Sample 4	25	11.57	0.68	5.90

Table 3: Intra-Assay-Variation

	Number of determinations	Mean value (µg/ml)	Standard deviation (µg/ml)	VC (%)
Sample 1	16	5.87	0.138	2.35
Sample 2	16	12.19	0.377	3.10
Sample 3	6	14.36	0.668	4.66

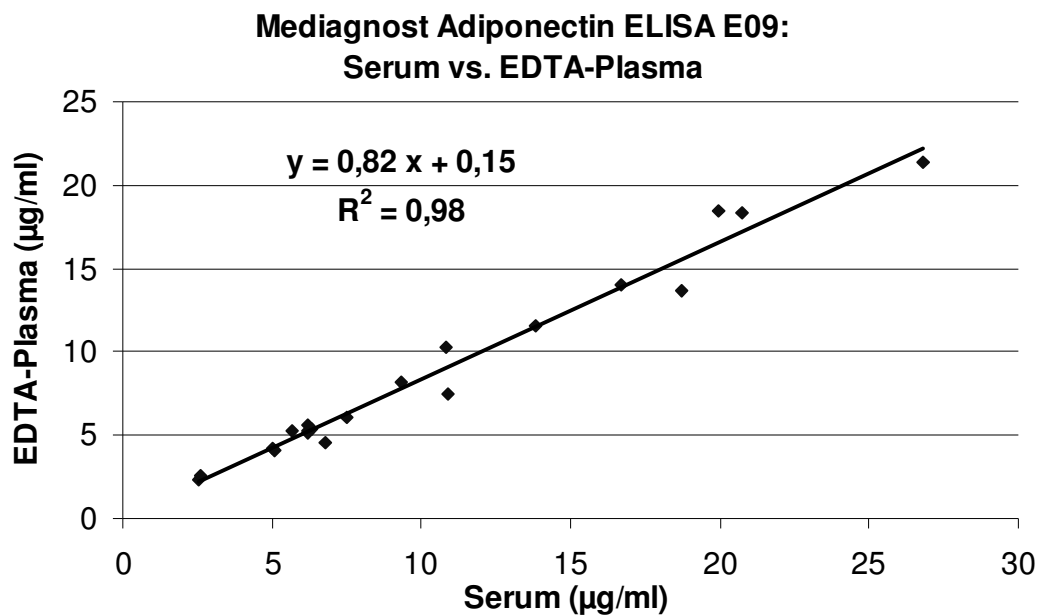
Samples: Applicability, Preparation and Storage

Serum as well as plasma samples are suitable (significant deviation of Adiponectin levels in corresponding Serum- and Heparinplasma samples were not found), in EDTA-Plasma-samples, as well as in citrat plasma samples levels were found approx. 20% lower. Due to the high correlation a conversion factor could be reasonable (s. Fig.1).

Common cell culture medium was found to be suitable. A special external sample pretreatment is not necessary.

Fig.1 : E09 - Adiponectin Values Serum vs. EDTA-Plasma:

20 different correspondent Serum- and EDTA-Plasma-samples respectively, were measured in 5 independent assay procedures in double determination.



Samples should be handled as recommended in general: as fast as possible and chilled as soon as possible. In case there will be a longer period between the sample withdrawal and determination, store the undiluted samples frozen at -20°C or below in tightly closable plastic tubes. Avoid on principle repeated freeze-thaw cycles of serum/plasma (if required, please subaliquote) although levels were found to be unaffected by few cycles.

The high sensitivity of the assay and the high serum concentration of Adiponectin allow measurement in small sample volumes, which is limited by pipetting accuracy rather than the amount of Adiponectin.

In most determinations (serum- or plasma samples and no extreme values expected) a dilution from **1:200 to 1:2000 with Dilution buffer VP** should be suitable. According to expected Adiponectin-levels the dilution with VP can be higher or lower. The Adiponectin-concentration may be completely different in body fluids of human origin other than serum or cell culture supernatants.

In general a dilution factor of **1:500 for serum or plasma samples** is optimally suitable for clinical diagnostics.

Suggestion for dilution protocol:

Dilute for example 490 µl Dilution Buffer VP in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 10 µl Serum- or Plasma (dilution: 1:50). Add **900µl** Dilution Buffer **VP** in an other PE-/PP-tube and **100µl** of the thoroughly mixed first dilution. After mixing, use **2×100 µl** from this **1:500** diluted sample in the assay.

MATERIALS

Materials provided

- 1) **Microtiter plate**, ready for use: Microtiter plate with **96 wells**, dived up in 12 stripes à 8 wells (**separately breakapart**), coated with anti-human Adiponectin antibody.
- 2) **Standards A-E**, lyophilised, contain native Adiponectin. Standard values are between **1-100 ng/ml** (1, 10, 30, 70 and 100 ng/ml) Adiponectin and have to be reconstituted in **750 µl (each) Dilution Buffer**. 100 µl per well are used in the assay. If the standards are required for more than one assay process we recommend to store the reconstituted Standards frozen at

-20 °C. **Attention:** Standards should be thawed only once – where required please store aliquoted in adequate volumes.

- 3) **Dilution buffer VP**, 120 ml, ready for use, please use for the reconstitution of Standards A-E, Control Serum KS and for the serum dilution.
- 4) **Control Serum KS**, lyophilised: Contains human Serum and has to be reconstituted in **100 µl Dilution Buffer VP**. The Adiponectin target value concentration and the respective range is given the vial label. The dilution of the Control Serum KS should be according to the dilution of the respective samples.
- 5) **Antibody-POD-Conjugate AK**, 12 ml, ready for use, contains a mixture of biotinylated anti-Adiponectin antibody and HRP (Horseradish Peroxidase)-labelled Streptavidin. Use 100 µl/well in the assay.
- 6) **Washing buffer WP**, 50 ml, 20-fold concentrated: Washing buffer has to diluted **1:20 with A.dest.** or demineralised water before use (e.g. add the complete contents of the flask **50 ml** into graduated flask and fill with A.dest to 1000 ml). Attention: After dilution, the Washing Buffer is only limited stable, please keep in cool place.
- 7) **Substrate S**, 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H₂O₂-Tetramethylbencidine.
- 8) **Stopping Solution SL**, 12 ml, ready for use, 0,2 M sulphuric acid, *Caution!*
- 9) **Sealing tape** for covering of the microtiter plate , 2 x, adhesive.

TECHNICAL RECOMMENDATIONS

In conducting the assay, follow strictly the test protocol.

Reagents with different lot numbers should not be mixed.

The Microtiter Plate and all unopened reagents are stable until the expiry date (s. label) if stored in the dark at 2° - 8°C.

For the **reconstitution** of the lyophilised components (**Standards A - E** and **Control Serum KS**) the kit **Dilution Buffer VP** should be used. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

The **shelf life of the components after opening** is not affected, if used appropriately.

Store the unused seal stripes of the microtiter plate together with the desiccant at 2° - 8°C.

Reconstituted components (Standards A – E and Control Serum KS) should be stored at -20°C (or below) for up to 2 months. **Avoid repeated freeze-thaw cycles.** In case you plan to perform multiple independent Adiponectin determinations over a longer period with one kit, you should aliquote the components prior to freezing into suitable smaller volumes. This is strongly recommended. The 1:20 diluted **Washing Buffer WP** is only limited stable. Please dilute only according to requirements.

Before use, all kit components should be brought to room temperature. Precipitates in buffers should be dissolved before use through mixing and warming.

Incubation at room temperature means: Incubation at 20 - 25°C.

The **Substrate Solution S**, stabilised H₂O₂-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

When **performing the assay**, the **Standards (A-E)**, **Control Serum (KS)** and **the samples** should be pipetted as fast possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times, **Antibody-POD-Conjugate AK** as well as the following **Substrate**

Solution S should be added to the plate in the same order and in the same time interval as the samples. **Stop Solution SL** should be added to the plate in the same order as the Substrate Solution.

Materials not Provided

- Distilled or demineralised water for dilution of the Washing Buffer WP
- Micropipettes and multichannel pipettes with disposable plastic tips
- Vortex-mixer
- Device to aspirate the standards and the samples from the wells (recommended because of the potential danger of infection by human Samples)
- Plate washer and plate shaker (recommended)
- Microplate reader ("ELISA-Reader") with filter for 450/620nm (or ≥ 590 nm).
- Foil welding device for laminate bags (recommended)

PROCEDURE

All determinations (Standards, Control and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

- 1) Add **100 μ l Dilution Buffer VP** in the wells A1/A2 (blank).
Pipette in positionen B1/2 **100 μ l of the Standard A (1 ng/ml)**
Pipette in positionen C1/2 **100 μ l of the Standard B (10 ng/ml)**,
Pipette in positionen D1/2 **100 μ l of the Standard C (30 ng/ml)**,
Pipette in positionen E1/2 **100 μ l of the Standard D (70 ng/ml)**,
Pipette in positionen F1/2 **100 μ l of the Standard E (100 ng/ml)**.

To control the correct accomplishment of the assay **100 µl** of the 1:500 (or in respective dilution ratio of the samples) diluted **Control Serum CS** can be pipetted in positions G1/2 .

Pipette **100 µl** each of the diluted sample (e.g. dilute 1:500 with Dilution Buffer VP) In the rest of wells, according to requirements.

- 2) Cover the wells with sealing tape and incubate the plate for **1 hours at room temperature** (if possible, shake at ≥ 350 rpm)
- 3) After incubation aspirate the contents of the wells and wash the wells 3 times **250 µl Washing Buffer WP** / well.
- 4) Following the last washing step pipette **100µl** of the **Antibody-POD-Conjugate AK** in each well.
- 5) Cover the wells with sealing tape and incubate the plate for **30 Minutes at room temperature** (if possible shake ≥ 350 rpm).
- 6) After incubation wash the wells 3 times with Washing Buffer WP as described in step 3.
- 7) Pipette **100 µl of the Substrate Solution S**.
- 8) Incubate the plate for **15 minutes in the dark at room temperature**.
- 9) Stop the reaction by adding **100 µl Stopping Solution SL** to all wells.
- 10) Measure the absorbance within **30 minutes at 450 nm (Reference filter ≥ 590 nm)**.

EVALUATION

Expected Values

Table 4: The expected values for Adiponectin were determined with the Mediagnost ELISA E09 in healthy donors and analysed by Prof. Dr. J. Kratzsch, Department of Laboratory Medicine, University Hospital Leipzig.

Female			Adiponectin (µg/ml):			
Age (Years):	n:	BMI: AV ± SD	AV ± SD::	Median :	Percentile: 25.- 75.	Min. – Max.:
Newborn Cord blood	19		29.80 ± 12.49	26.1	19.5-35.2	16.9-61.4
< 3.99	9	15.73 ± 0.79	14.43 ± 7.76	11.2	8.2-21.8	2.3-26.7
4.0-7.99	11	16.01 ± 1.94	8.46 ± 4.73	9.3	2.9-12.1	1.4-15.6
8.0-9.99	22	17.58 ± 3.84	7.92 ± 3.00	8.2	5.2-10.0	3.6-15.1
10.0-11.99	33	17.83 ± 1.86	7.66 ± 4.59	6.6	5.0-8.8	3.1-20.9
12.0-13.99	11	19.85 ± 2.31	8.22 ± 5.64	7.5	6.5-9.2	4.9-13.2
14.0-15.99	27	19.91 ± 1.72	8.83 ± 9.25	8.9	5.2-11.8	2.6-17.7
16.0-19.99	18	21.64 ± 2.64	9.00 ± 3.22	8.7	6.9-11.2	2.7-14.0
20.0-29.99	24	23.12 ± 5.01	7.39 ± 3.35	7.3	5.7-9.0	3.4-17.8
30.0-39.99	17	23.20 ± 2.86	9.19 ± 3.89	8.6	7.2-10.4	3.6-19.3
40.0-49.99	26	24.50 ± 4.11	9.93 ± 3.59	9.5	7.5-11.6	4.4-19.6
50.0-59.99	21	24.61 ± 3.31	11.5 ± 5.49	10.0	8.0-15.9	2.0-23.1
>60.0	8	24.63 ± 1.89	15.6 ± 4.64	15.3	11.4-18.2	11.2-24.1

n= Number of Proband **AV**=Average Value, **BMI**=Body Mass Index (kg/m²) **SD**=Standard Deviation

Male			Adiponectin ($\mu\text{g/ml}$):			
Age (Years):	n:	BMI: AV \pm SD	AV \pm SD:	Median :	Percentile: 25.- 75.	Min. – Max.:
Newborn Cord blood	10		27.80 \pm 7.68	26.7	22.2-31.0	15.6-40.6
< 3.99	14	16.17 \pm 1.81	16.57 \pm 6.55	14.3	11.6-21.2	5.8-40.3
4.0-7.99	12	15.69 \pm 1.05	11.24 \pm 5.43	9.7	8.9-15.9	3.5-18.6
8.0-9.99	18	16.45 \pm 1.76	8.11 \pm 2.93	7.6	6.2-9.1	5.00-15.4
10.0-11.99	21	18.34 \pm 2.18	8.43 \pm 3.91	7.8	5.2-10.9	3.4-20.2
12.0-13.99	14	18.61 \pm 2.11	7.59 \pm 2.86	7.1	6.0-10.3	2.4-12.2
14.0-15.99	32	19.86 \pm 2.00	7.53 \pm 2.52	7.4	5.1-9.3	3.8-15.4
16.0-19.99	23	22.03 \pm 2.42	7.16 \pm 3.53	6.9	4.2-9.6	2.0-13.9
20.0-29.99	23	23.43 \pm 2.48	5.44 \pm 2.29	5.8	4.0-6.9	1.3-10.3
30.0-39.99	21	23.33 \pm 2.72	5.92 \pm 4.60	4.4	2.7-6.7	1.9-20.6
40.0-49.99	22	23.79 \pm 2.41	6.13 \pm 2.92	5.5	3.8-8.3	2.1-11.6
50.0-59.99	23	26.68 \pm 2.77	7.45 \pm 4.50	6.7	5.0-8.8	1.4-19.6
>60.0	24	25.72 \pm 2.12	7.48 \pm 3.92	7.6	4.6-9.2	3.0-21.1

n= Number of Proband **AV**=Average Value, **BMI**=Body Mass Index (kg/m^2) **SD**=Standard Deviation

These data show significant correlation between Adiponectin-Serum values and age of the probands, in turn the correlation between the respective BMI seems to be less significant. In the samples of neonatal cord blood very high values were found.

Establishing the Standard Curve

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.25, these of standard E should exceed 1.0.

Samples, which yield higher absorbance values than Standard E are beyond the standard curve, for reliable determinations these samples should be tested anew with a higher dilution.

The standards provided contain the following concentrations of Adiponectin:

Standard	A	B	C	D	E
ng/ml	1	10	30	70	100
µg/ml	0.001	0.01	0.03	0.07	0.1

Establishing the Standard Curve

- 1) Calculate the mean absorbance (MA) value for the blank from the duplicated determination (well A1/A2).
- 2) Subtract the means absorbance of the blank from the mean absorbances of the standards and of the samples.
- 3) Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
- 4) Recommendation: Calculation of standard curve should be done by using a computer programme, because the curve is in general (without respective transformation) not ideally described by linear regression. **Non-linear-regression**, a higher-grade **polynomial** or **four parameter logistic (4PL)** lin-log curve fit are suitable for the evaluation.
- 5) The Adiponectin concentration of the sample in ng/ml (or in µg/ml according the chosen unit for the standards) can be calculated by multiplication with the respective dilution factor.

PRECAUTIONS

General

All reagents are for in vitro use only!

The acquisition, possession and use of the kit are subjects to the regulations of the national regulatory authorities.

Reagents contain as preservative Thimerosal, however, highly diluted (0.02%). Thimerosal is toxic when swallowed and it involves a certain danger of cumulative effects (R-Phrases 26/27/28-33-50/53 and S 13-28.1-36-45-60-61).

First aid procedures:

Skin contact: Wash affected area thoroughly with water. Discard contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.

Ingestion: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.

The Stop Solution SL provided is an acid solution. Avoid direct contact. Wear eye, hand, face and clothing protection when using this material.

The handling of potentially infectious human material (in the test kit only the provided Control Serum KS, has been shown negative for HBsAg, anti-HIV-1 and -2 and the individual samples) must comply with the following guidelines:

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

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SUMMARY OF THE ASSAY

Reagent preparation:	Reconstitution:	Dilution
Standards A-E	in 750 µl Dilution Buffer VP	
Control Serum KS	in 100 µl Dilution Buffer VP	1:500 with Dilution Buffer VP
Washing Buffer WP		1:20 with Aqua. dest. (e.g., add the complete contents of the flask (50 ml) into a graduated flask and fill with A.dest. to 1000 ml).
<p>Sample Dilution: Dilute for example 490 µl Dilution Buffer VP in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 10 µl Serum- or Plasma (dilution: 1:50). Add 900µl Dilution Buffer VP in an other PE-/PP-tube and 100µl of the thoroughly mixed first dilution. After mixing, use 2×100 µl from this 1:500 diluted sample in the assay.</p>		

Assay Procedure for Double Determination:

Pipette	Reagents	Position
100 µl	Dilution Buffer VP	A1/2
100 µl	Standard A (1 ng/ml)	B1/2
100 µl	Standard B (10 ng/ml)	C1/2
100 µl	Standard C (30 ng/ml)	D1/2
100 µl	Standard D (70 ng/ml)	E1/2
100 µl	Standard E (100 ng/ml)	F1/2
100 µl	Control Serum KS	G1/2
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
Incubation: 1 h at RT, ≥ 350 rpm		
3x 250 µl	Aspirate the contents of the wells and wash 3x with 250 µl Wash Buffer WP	each well
100 µl	Antibody-POD-Conjugate AK	each well
Incubation: 30 min at RT, ≥350 rpm		
3x 250 µl	Aspirate the contents of the wells and wash 3x with 250 µl Wash Buffer WP.	each well
100 µl	Substrate Solution S	each well
Incubation: 15 min in the Dark at RT		
100 µl	Stopping Solution SL	each well
Measure the absorbance within 30 min at 450 nm with ≥590 nm as reference wavelength.		