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Mouse Adiponectin ELISA

Enzyme Immunoassay for the Quantitative Determination of
Mouse Adiponectin
English

for research use only
Not for use in diagnostic procedures.
for professional use!

2-8°C 96 wells

h **E091-M**

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PACKAGE INSERT ENGLISH

Mediagnost Maus Adiponectin ELISA E091-M

- is suited for Adiponectin determination in **Mouse Serum** and **Plasma** samples
- is **fast**: incubation time a total of 2 hours and 30 minutes
- Single Standards with **0.025/ 0.075/ 0.15/ 0.3/ 0.65 and 1 ng/ml** native Mouse-Adiponectin are provided in the Kit
- 2 Control Sera are provided for quality control
- uses **high affinity monoclonal antibodies** against mouse Adiponectin
- Microtiter plates are separately breakapart

Intended Use

Measurement of Adiponectin in Mouse Serum and Plasma samples.

Introduction

Adiponectin was described for the first time in the early 90th of the last century as an endocrine factor produced by adipocytes. Adiponectin is involved in regulation of energy- and fat metabolism. So its concentration in the circulation is said to reflect the risk of atherosclerosis and the degree of insulin resistance. Based on the high incidence of these diseases, adiponectin was and still is object of intensive research regarding the underlying biological mechanisms and regarding its value as biomarker. Beside different cell culture models and studies with human patients, mice and rats are suitable model organisms for basic research and pre-clinical studies.

Therefore we developed and validated this testsystem as a tool for adiponectin measurements in mice usable in research and pre-clinical studies.

Even if the comparability of mice and humans is limited we offer some background information on *human* adiponectin physiology in the following section:

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 ditrimer also high molecular multimers exist [1-3]. Up to now two different receptors are known, both receptors are ubiquitary 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 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].
The formerly proposed diagnostic value of the high molecular weight form of adiponectin was not verified using a commercially available testsystem for the determination of HMW adiponectin [10]. Blueher et al. clearly demonstrate that regarding the diagnosis of insulin resistance, measured by whole body glucose uptake below 40 µmol/kg*min, total adiponectin as determined with the Mediagnost E09, is with an area of 0.92 under the receiver-operating curve, of greater diagnostic value [10].

Furthermore it is involved in inflammatory processes [11-15] and therewith it is of importance for appearance of arteriosclerosis [4, 5, 16] and coronay disease [17, 18], thus the determination of Adiponectin level in plasma could serve to estimate the risk of coronary disease [19, 20]. Beside this Adiponectin influences further physiological processes as for example the angiogenesis [21, 22].

Reagents Provided

1)	MTP	**Microtiter plate**, ready for use: Microtiter plate with 96 wells, divided up in 12 stripes à 8 wells (separately breakable), coated with anti-Maus Adiponectin antibody.
2)	STD	**Standards A-F**, lyophilised, contain native Mouse Adiponectin. Standard values are between **0.025 - 1 ng/ml** (0.025, 0.075, 0.15, 0.3, 0.65 und 1 ng/ml) Adiponectin and have to be reconstituted in **1 ml (each) Dilution Buffer VP**. 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)	DILU	**Dilution buffer VP**, **125 ml**, ready for use, please use for the reconstitution of Standards A-F, Control Sera KS1 & KS2 and for the serum dilution.
4)	Control	**Control Serum KS1 & KS2**, each **250 µl** lyophilised: Contains mouse Serum and has to be reconstituted in **250 µl Dilution Buffer VP**. The Adiponectin target value concentration and the respective range is given on the certificate. The dilution of the Control Sera KS 1&2 should be according to the dilution of the respective samples, the target value concentration should be obtained by **multiplication** with the respective **dilution factor**.
5)	Ab CONJ	**Antibody-HRP-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)	WASHBUF 20x	**Washing Buffer (WP), 50 ml, 20 X concentrated** solution. **Washing Buffer (WP)** has to be diluted 1:20 with distilled or demineralised water before use (e.g. add the complete contents of the flask (50 ml) into a graduated flask and fill up with A.dest. to 1000 ml). Attention: After dilution the Washing Buffer is only 4 weeks stable, dilute only according to requirements.
7)	SUBST	**Substrate (S), 12 ml,** ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised Tetramethylbencidine.
8)	H_2SO_4	**Stopping Solution (SL), 12 ml,** ready for use, 0.2 M sulphuric acid, Caution acid!
9)		Sealing tape for covering of the microtiter plate, 2 x, adhesive.

Materials Required but not provided

Precision pipettes and multichannel pipettes with disposable plastic tips
Distilled or deionized water for dilution of the Washing Buffer (WP)
Vortex-mixer

Microtiterplate shaker (350 rpm)
Microtiterplate washer (recommended)
Micro plate reader ("ELISA-Reader") with filter for 450 and ³ 590 nm
Polyethylen PE/Polypropylen PP tubes for dilution of samples

WARNINGS AND PRECAUTIONS

For in-vitro use only. For professional use only.
Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. Safety Data Sheet is available on request.

Before use, all kit components should be brought **to room temperature at 20 - 25°C**. Precipitates in buffers should be dissolved before use by thorough mixing and warming.

Do not mix reagents of different lots. Do not use expired reagents.

The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.

Caution: This kit contains material of animal origin.

No known test methods can offer total assurance of the absence of infectious agents; therefore all components and specimens should be treated as potentially infectious.
Do not use obviously damaged or microbial contaminated or spilled material.

Caution: This kit contains material of animal origin. Therefore all components and specimens should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations

Mouse Serum contained in the following components: **KS1 and KS2, STD A-F**

A –F, AK, VP, WP contain as preservatives 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (<0.015 %)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

The Stop solution contains 0.2 M acid sulphur acid (H_2SO_4)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+	IF SWALLOWED: rinse mouth.
P331	Do NOT induce vomiting.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step. Use separate pipette tips for each sample, control and reagent to avoid cross contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine. Store and Incubate in the dark.

Substrate Solution (S)
The TMB-Substrate (S) contains 3.3´,5.5´ Tetramethylbencidine

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.

General 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.
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.

Method
The Mediagnost ELISA for Adiponectin E091-M 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.

Specimen
Serum and plasma samples of mice and mouse cell culture medium can be used in this assay.

Influence of Heparin (30 IE/mL), EDTA (6,8 mM) and NaCitrat (0,015 M) on the measurement of Adiponectin has been investigated be recovery experiments. PBS was enriched with recombinant mouse Adiponectin and the above-mentioned substances. No significant influence on the recovery of adiponectin was detected.
Hemolytic reactions have to be avoided. The blood has to be allowed to clot and after complete clotting, serum is separated by centrifugation.

Storage of the samples

Storage at RT max. 2 days

Storage at –20°C max. 2 years

Do not perfom more than five froze / thaw cycles.

Sample Preparation
Samples have to be diluted in Dilution Buffer (VP).
A sample dilution of 1:10 000 is in general suitable. However, the Adiponectin levels can vary individually significant, we would therefore recommend to check this and adjust the dilution respectively.

Technical Recommendations

The assay has to be conducted strictly according the test protocol herein.

Reagents with different lot numbers cannot be mixed. The microtiterplate and reagents are stable until the indicated expiry if stored unopened and protected from sunlight at 2 – 8°C.

Storage Life
The shelf life of the components after initial opening is warranted for 4 weeks, store the unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The reconstituted components standards A-F and Control Sera KS1 and KS2 must be stored at –20°C (max. 4 weeks).
Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming.

Incubation at room temperature means: Incubation at 20-25°C
The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical

differences deviations may occur, in case the rotation frequency must become adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variations and/or false values, excessive shaking may result in high optical densities and/or false values.
Proper washing is of basic importance for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.

All washing must be performed with the provided washing buffer diluted to usage concentration. Washing volume per washing cycle and well must be 300 µl at least.

The danger of handling with potentially infectious material must be taken into account.

When using an automatic microtiter plate washer, the respective instructions fur use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non fuzzy absorbent tissue.

Manual washing is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamically swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non fuzzy absorbent tissue.

Standards and Controls

For the reconstitution of the lyophilised components (Standards A - F and Control Sera KS1 &KS2) the kit Dilution Buffer VP has to 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!) with a Vortex mixer.

The reconstituted standard and controls can be stored for 4 weeks at –20°C. Repeated freeze/thaw cycles have to be avoided.

Washing Buffer

The required volume of washing buffer is prepared by 1:20 dilution of the provided 20fold concentrate with deionised water. The diluted Washing Buffer is stable for 4 weeks at 2-8°C. It has to be at room temperature for usage!

Microtiter plate

Unused microtiter plate stripes have to be stored airtight together with the desiccant bag at 2-8°C.

Assay Procedure
All determinations (Standards, Control Sera KS1 & KS2 and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

When performing the assay, the Standards, Control Sera and the samples should be pipetted as fast as possible (e.g., <15 minutes).

All incubations have to conducted at room temperature (20-25°C)

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.

1) Add **100 µl Dilution Buffer VP** to wells A1 and A2 (Blank).
Add 100 µl standard or 100 µl diluted control sera or diluted samples to the appropriate wells.

2) Cover the wells with sealing tape and incubate the plate for **1 hour** at **room temperature** (shake at 350 rpm)

3) After incubation aspirate the contents of the wells and wash the wells **5 times 300 µl Washing Buffer WP** / well. The washing buffer should incubate for at least for 15 seconds/cycle.

4) Following the last washing step pipette **100 µl** of the **Antibody-POD-Conjugate AK** in each well.

5) Cover the wells with sealing tape and incubate the plate for **1 hour** at **room temperature** (shake at 350 rpm).

6) After incubation wash the wells **5 times** with Washing Buffer as described in step 3

7) Pipette **100 µl of the TMB Substrate** Solution in each well.

8) Incubate the plate for **30 minutes in the dark at room temperature (20 - 25°C)**.

9) Stop the reaction by adding **100 µl of Stopping Solution**.

10) Measure the colour reaction within 30 minutes at **450 nm** (**reference filter ≥590 nm**).

Calculation of Results

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 F should be above 1.0.

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

Standards are provided in the following concentrations (use the concentration unit as preferred):

Standard	**A**	**B**	**C**	**D**	**E**	**F**
ng/ml	0.025	0.075	0.15	0.3	0.65	1

1) Calculate the **mean absorbance** value for the blank from the duplicated determination (well A1/A2).

2) Subtract the mean absorbance of the blank from the mean absorbances of all other values.

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 the standard curve should be done by using a computer program. **A higher-grade polynomial,** or **four parametric logistic (4-PL) curve fit** or **non-linear regression** are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).

5) The adiponectin concentration of the diluted controls KS1 & KS2 or the diluted samples is obtained from the standard curve. The multiplication of the respective calculated adiponectin content by the corresponding dilution factor then results in the adiponectin concentration of the undiluted starting solutions.

Performance Characteristics

Standards

The Standards of the ELISA E091-M are prepared from **native Adiponectin** in concentrations of **0.025/ 0.075/ 0.15/ 0.3/ 0.65 and 1 ng/ml**. The native Adiponectin was calibrated against the recombinant Protein (Manufacturer: R&D Systems, Wiesbaden).

Sensitivity

The analytical sensitivity of the ELISA E091-M yields 0.008 ng/mL (equal to < 0.0008 ng per well; as 2xSD of zero standard in 22fold replicates).

Interassay Variability

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Mean	1.12	6.12	7.65	8.04	2.15	7.54	6.87
SD	0.09	1.19	0.60	0.56	0.25	0.55	0.42
VC%	7.66	3.79	7.80	6.98	11.80	7.28	6.08
n	8	8	9	9	9	9	6

Intraassay Variability

	Sample 1	Sample 2
Mean [µg/mL]	7.395	1.712
SD	159	37
VC%	2.15	2.16
n	21	21

Linearity

Linearity				
Dilution	**Sample 1**	**Dilution**	**Sample 2**	**Sample 3**
1:14000	11.673	1:10000	9.59	
1:28000	10.928	1:12000	11.229	11.886
1:56000	10.67	1:24000	9.856	10.822
1:112000	10.421	1:48000	9.915	10.222
		1:96000	9.684	9.999

recalculated Values in ng/mL

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SUMMARY – MEDIAGNOST Mouse Adiponectin ELISA E091-M

Reagent preparation:	**Reconstitution:**	**Dilution**
Standards A-F	in 1 ml Dilution Buffer **VP**	
Control Sera KS1 & KS2	in 250 µl Dilution Buffer **VP**	**1:10 000 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).
Sample Dilution: e.g. 1 :10 000		

Assay Procedure for Double Determination:

Pipette	**Reagents**	**Position**
100 µl	Dilution Buffer **VP**	A1/2
100 µl	Standard **A** (**0.025 ng/ml**)	B1/2
100 µl	Standard **B** (**0.075 ng/ml**)	C1/2
100 µl	Standard **C** (**0.15 ng/ml**)	D1/2
100 µl	Standard **D** (**0.3 ng/ml**)	E1/2
100 µl	Standard **E** (**0.65 ng/ml**)	F1/2
100 µl	Standard **F** (**1 ng/ml**)	G1/2
100 µl	Control Serum **KS1**	H1/2
100 µl	Control Serum **KS2**	A3/4
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
Incubation: 1 h at RT, 350 rpm		
5x 300 µl	Aspirate the contents of the wells and wash 5x with **300 µl** Wash Buffer **WP**	each well
100 µl	Antibody-HRP**-**Conjugate **AK**	each well
Incubation: 1 h at RT, 350 rpm		
5x 300 µl	Aspirate the contents of the wells and wash 5x with **300 µl** Wash Buffer **WP**	each well
100 µl	Substrate Solution **S**	each well
Incubation: 30 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.		

REF E091-M  **International Test description**

STD	**A -F**	**Rec in** 1 ml **VP**	
Control	**KS1 & KS2**	**Rec in** 250 µl **VP**	**1:10 000 DILU VP**
WASHBUF 20x	**WP**		**1:20 DILU A. dest.**
SPE			**1:10 000 DILU VP**
°C 20-25 °C			

100 µl	**VP**	A1/2
100 µl	**STD A (0.025 ng/ml)**	B1/2
100 µl	**STD B (0.075 ng/ml)**	C1/2
100 µl	**STD C (0.15 ng/ml)**	D1/2
100 µl	**STD D (0.3 ng/ml)**	E1/2
100 µl	**STD E (0.65 ng/ml)**	F1/2
100 µl	**STD F (1 ng/ml)**	G1/2
100 µl	**CONTROL KS1 1:10 000 DILU VP**	H1/2
100 µl	**CONTROL KS2 1:10 000 DILU VP**	A3/4
100 µl	**SPE 1:10 000 DILU VP**	
TAPE		

À 1 h °C 20-25 ↔ 350 rpm

5x 300 µl	**5x WASHBUF WP**
100 µl	**AbCONJ AK**
TAPE	

À 1 h °C 20-25 ↔ 350 rpm

5x 300 µl	**5x WASHBUF WP**
100 µl	**SUBST TMB S**

À 0.5 h °C 20-25

100 µl	**H_2SO_4 SL**
MEASURE	