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Rat Adiponectin ELISA

Enzyme Immunoassay for quantitative Determination of
Rat Adiponectin
English

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REF **E091-R**

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Symbols/ Symbole /Symboles/ Simboli/ Símbolos/ Símbolos/ symbolen/ Symboler/ Symboler/Symbole/ Szimbólumok/ Symboly/ Symboly/ Символи/ Sümboldid/ Σύμβολα/ Simboluri/ Simboli/ Symbolit

according to DIN EN 980 and EDMA recommendations Standard News 6 2001



Expiry date/ Verfallsdatum/ Date de péremption/ Data di scadenza/ Fecha de caducidad/ Data de validade/ Uiterste gebruiksdatum/ Udløbsdato/ Bäst före-datum/ Termin ważności/ Lejárati idő/ Čas expirácie/ Doba expirace/ Срок на годност/ Αεγυμίσκυρπäv/ Ημερομηνία λήξης/ Data de expirare/ Rok uporabe/ Viimeinen käyttöpäivä



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Lot-Batch Number/ Charge-Chargennummer/ Lot-Code du lot/ Lotto-Numero di lotto/ Lote-Código de lote/ Lote-Código do Lote/Lot-Partijnummer/ Lot-Batchkode/ Partisatskod/ Numer serii/ Tétel-sarzs szám/ Číslo šarže/ Číslo šarže/ Партиден номер/Partii – partii number/ Παρτίδα-αριθμός παρτίδας/ Lot-număr lot/ Številka serije/ erä



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Store at between/ Lagerung bei zwischen/ Conserver à entre/ Conservare a tra/ Conservar a temp. Entre/ Armazenar entre/ Bewaar bij tussen/ Opbevares mellem/ Förvaras vid/ Przechowywać w/ Tárolási tartomány/ Skladujte v rozsahu / Skladujte v rozmezí/ Температурно ограничение/ Säililitada temperatuuridel/ Φύλαξη σε θερμοκρασία/ Depozitare între/ Skladiščenje med/ Säilytys x-y Celsiusasteen lämpötilassa



Contains sufficient for x tests/ Inhalt ausreichend für x Tests/ Contient suffisant pour x tests/ Contenuto sufficiente per x test/ Contenido suficiente para x pruebas/ Conteúdo suficiente para x testes/ Bevat voldoende voor x bepalingen/ Indeholder tilstrækkeligt til x prøver/Innehållet räcker till x analyser/ Zawartość na x testów/ Tartalma x teszt elvégzésére elegendő/ Obsahuje materiál pre x testov / Obsah dostačuje pro x testů/ Съдържание достатъчно за x тестове/ Sisust jätkub x katse jaoks/ Το περιεχόμενο επαρκεί για x δοκιμές/ Conținut suficient pentru x teste/Vsebina zadostuje za x preizkusov/ Sisältö riittää x testille



Keep away from sunlight/ Nicht dem Sonnenlicht aussetzen/ Conserver à l'abri de la lumière/ Conservare al riparo della luce solare/ No exponer a la luz solar/ Protéger da luz solar/ Niet aan zonlicht blootstellen/ Må ikke udsættes for sollys/ Utsatt inte för solljus/ Nie wystawiać na słońce/ Napfénytől távol tartandó/ Nevystavovat slnečnému svetlu/ Nevystavovat slunečnímu světlu/ Да се предпазва от слънчева светлина/ Kaitsta otsese päikesekiirguse eest/ Κρατήστε το μακριά από την ηλιακή ακτινοβολία/ Țineți departe de lumina soarelui/ Ne izpostavljajte sončni svetlobi/ suojaa auringonvalolta



Incubation time/ Inkubationszeit/ Temps d'incubation/ Tempo d'incubazione/ Tiempo de incubación/ Tempo de incubação/ incubatietijd/Inkubationstid/ inkubationstid/ Czas inkubacji/ Inkubációs idő/ Inkubačná lehota/ Inkubační doba/ Инкубационен период/ Inkubatsiooniaeg/ Χρόνος επώασης/ Timp de incubare/Inkubacijska doba/ inkubaatioaika



incubate at / Inkubation bei/ Incuber à/Incubare a/incubar a/Incubar a/incubatietemperatuur/Inkubation ved/inkubation vid/Inkubacja przy/Inkubáció hőmérséklete/Inkubácia pri/Inkubace při/Инкубира се при/Inkubatsioon temperatuuril/Επώαση στους/Incubare la/Inkubacija pri/ inkubaatiolämpötila



Shaking/ Schütteln/ Mélanger/ Agitare/ Agitar/ Agitação/ Schudden/ Ryster/ Skaka/ Wstrząsanie/ Rázás/ Pretreat/ Protřepat/ Разклатяне/ Raputada/ Ανακινήστε/ Vibrare/ Stresite/ Sekoita



Mikrotiterplate/ Mikrotiterplatte/ plaque de microtitrage/ Piastra di microtitolazione/ Placa de microtitulación/ Placa de Microtitulaço/ microtiterplaat/ Mikrotiterplade/ mikrotiterplatta/ microtiterplaat/ Płytko microtiter/ Mikrotiter lap/ Mikrotitračná podložka/ Mikrotitrační podložka/ Микротитърна плака/ Mikrotiiterplaat/ Τρυβλίο μικροπιλοδότησης/ Microplacă/ Mikrotitrská plošča/ Mikrotitruslevy



Reconstitute in/ Rekonstituieren in/ Reconstituer dans/ Ricostituire nel/ Reconstituier en/ Reconstituier em/ reconstituieren in/ Rekonstituér i/ rekonstituera/ Rekonstytiować w/ Helyreállítás/ Znovu pripravit' za/ Znovu připravit za/ Разтваряне в/ Moodustada uuesti / Ανασυστήστε σε/ Reconstituire în/ Predelava v /rekonstituoi



Sample/ Probe /Echantillon/ campione/ Muestra/ Amostra/ monster/ Prøve/ prov/ Próbka/ Minta/ Vzorka/ Vzorek/ Проба/ Proov/ Δείγμα/ Probă/Vzorec/Näyte



Antibody and Enzyme Conjugate/ Antikörper und Enzym Konjugat/ anticorps conjugué et conjugué enzymatique/ Coniugato di anticorpo ed enzima/ Conjugado de anticuerpos y enzimas/ Conjugado Anticorpo-Enzima/ antilichaam- en enzymconjugaat/ Antistoffer og enzym-konjugat/ antikropp- och enzymkonjugat (antikropp och enzym, konjugat)/ Koniugat antyciał i enzymów/ Antitest és enzim páros/ Protílátkový a enzymatický konjugát/ Protílátkový a enzymatický konjugát/ Антитяло и ензим конюгат/ Antikehad ja ensüümi konjugaat/ Σύμπλοκο αντισώματος-ενζύμου/ Compuși din anticorpi și enzime/ Antitelesa in konjugat encima/ Vasta-aine ja entsymi konjugaatti



Dilute in Buffer X/ Verdünnen in Puffer X/ Diluer dans le tampon X/ Diluire nel tampone X/ Diluir en tampón X/ Diluir no Tampão X/ verdunnen in buffer X/ Fortyndes i buffer X/ späd i buffert X/ Rozcieńczenie w buforze X/ Hígítás X pufferben/ Riedit' v pufri X/ Ředit v pufru X/ Разреждане в буфер X/ Lahjendada puhvriss X/ Αραιώστε σε ρυθμιστικό διάλυμα X/ Diluati în tamponul X/ Razredčiti v pufru X/ laimennetaan x puskuriin



Standard X /Standard X/ Etalon X/ Standard X/ Estándar X/ Standard X/ standaard X/ Standard X/ standard X/ Standard X/ Standard X/ Стандарт X/ Standard X/ Πρότυπο X/ Standard X/ Standardni X/ Kalibraattori X

Control	KS1 Control Serum / Kontrollserum/ Contôle sérique/ Siero di controllo/ Suero de control/ Soro de Control/ / controleserum/ Kontrolserum/ Kontrollserum/ Serum kontrolne/ Ellenőrző szérum/ Kontrolné sérum/ Kontrolní sérum/ Контролен сeрyм/ Kontrollseerum/ Ορός ελέγχου/Ser de control/ Kontrolni serum/ Kontrolli seerumi
WASHBUF 20x	WP Washing Buffer Concentrate/ Waschpufferkonzentrat/ Tampon de lavage conc./ Tampone di lavaggio concentrato/ Tampón de lavado concentrado/ Tampão de Lavagem Concentrado/ wasbuffer, geconcentreerd/ Vaskebufferkoncentrat/ Vaskebufferkoncentrat/ tvättbuffertkoncentrat/ Bufor płukania koncentrat/ Mosópuffer koncentrátum/ Koncentrát vymývacieho pufra/ Концентрат на промивен буфер/ Pesupuhvri kontsentraat/ Συμπύκνωμα ρυθμιστικού διαλύματος έκπλυσης/ Concentrat pentru tamponul de spălare/ Koncentrat izpiralnege pufra/ Pesuliositiiviste
WASHBUF	Washing Buffer / Waschpuffer/ Tampon de lavage/ Tampone di lavaggio/ Tampón de lavado/Tampão de Lavagem/ wasbuffer/ Vaskebuffer/ tvättbuffert/ Bufor płukania/ Mosópuffer/ Vymývací pufer/ Vymývací pufr/ Промивен буфер/ Pesupuhver/ Ρυθμιστικό διάλυμα έκπλυσης/ Tampon pentru spălare /Izpiralni pufer/ Pesuliuos
SUBST TMB	S Substrate/ Substrat/ Substrat/ Substrato/ Substrato/ Substrato/ substraat/ Substrat/ Substrat/ Substrat/ Szubstrátum/ Substrát/ Substrát/ Субстрат Substraat/ Υπόστρωμα/ Substrat/ Substrat/ Substraattiliuos
H₂SO₄	SL Stop Solution/ Stopp Lösung/ Stop Solution/ Soluzione di stop/ Stop Solución/ Solução Stop/ stopoplossing/ Stopopløsning/ Stopplösning/ Stop roztwór/ Megállító oldat/ Roztok na ukončenie/ Roztok pro ukončeni/ Стопирач разтвор/ Stopp-lahus/ Διάλυμα διακοπής/ Soluție de oprire/ Stop raztopina/ Pysäytysliuos
TAPE	Cover Plate with sealing tape /Platte abkleben/ Recouvrir la microplaque avec bande adhésive/ Coprire la piastra con nastro adesivo/ Cubrir la placa con una cinta adhesiva/ Cobrir a Placa com fita adesiva/ plaatje met tape afdekken/ Afdækningsplade med tape/ maskera platta/ Odkleić plytkę/ Tányér leragasztása/ Oblepiť podložku lepiacou páskou/ Olepiť podložku lepící páskou/ Пластика с лента за запечатване/ Katta plaat isoleerkiieplindiga/ Κολλήστε το πλακίδιο με κολλητική ταινία/ Aoperiți placa cu o bandă adezivă/ Prelepiti ploščo/ Peitã mikrotitrauslevy oheisella teipillä
MEASURE	Measure plate within 30 min at 450 nm (Referencefilter ≥590nm)/Ausmessung innerhalb von 30 min bei 450 nm (Referenzfilter ≥ 590 nm)./ Mesure l'absorbance en l'espace de 30 min à 450 nm avec ≥590nm longueur d'onde pour référence/Misurazione entro 30 min. a 450 nm (filtro di riferimento ≥ 590 nm)./ Medición de la placa dentro de los siguientes 30 min a 450 nm (filtro de referencia ≥ 590nm)/ Medir a placa dentro de 30 min a 450 nm (Filtro de referência ≥590nm)/ Binnen 30 minuten bij 450 nm meten (referentiefilter ≥ 590 nm)./ Mål plade i løbet af 30 min ved 450 nm (referencefilter ≥590nm)/ Mät inom 30 min vid 450 nm (referensfilter ≥ 590 nm)./ Pomiar w ciągu 30 min przy 450 nm (filtr odniesienia ≥ 590 nm)./ Ki mérés 30 percen belül 450 nm-nél (referenciaszűrő ≥ 590 nm)./ Merat' 30 minut pri 450 nm/Měřit 30 minut při 450 nm/ Отчитане в рамките на 30 min при 450 nm (референтен филтър ≥ 590 nm)./ Mõõtmise 30 min jooksul 450 nm korral (võrdlusfilter ≥ 590 nm)./ Μέτρηση εντός 30 min στα 450 nm (φίλτρο αναφοράς ≥ 590 nm)./ Măsurare în decurs de 30 min la 450 nm (filtru de referință ≥ 590 nm)./ Izmerite ploščico v 30 min pri 450 nm (referenčni filter ≥590nm) / Mittaa 30 minuutin aikana 450 nm:ssä (referenssi suodatin ≥ 590 nm)
Literatur	Literature/ Literatur/ Bibliographie/ Letterario/ Bibliografia/ Literatura documentaçaõ/ literatuur/ Litteratur/ litteratur/ Literatura/ Irodalom/ Literatúra/ Literatura/ Литература/ Kirjandus/ Βιβλιογραφία/ Bibliografie/ literatura/ Lähdeluettelo
International Test description	International test description/ internationale Testanleitung/ description internationale de test/ Istruzioni per il test internazionali/ Descripción de ensayo internacional/ Descrição internacional do teste/ internationale testbeschrijving/ internationell testbeskrivning/ Opis testu międzynarodowego/ nemzetközi teszt-útmutató/ Medzinárodný návod k testu/ Mezinárodní návod k testu/ rahvusvaheline katse kirjeldus/ Διεθνείς οδηγίες για εργαστηριακές δοκιμές/ instrucțiuni internaționale pentru testare/ mednarodna navodila za preizkus/ Kansainvälinen käyttöohje
End	in all required wells/ in allen benötigten Vertiefungen/ dans tous les godets requis/ in tutti i pozzetti richiesti/ en todos los pozos requeridos/ em todos os tubos necessários/ in alle nodige putjes/ i alle nødvendige brønde/ i alla nödvändiga brunnar/ we wszystkich potrzebnych wgłębieniach/ minden szükséges forrásban/ vo všetkých potrebných miestach/ ve všech potřebných místech/ във всички необходими ямки/ kõigis vajalikes süvendites/ σε όλες τις απαραίητες κοιλότητες/ în toate cavitățile necesare/ v vseh zahtevanih vdolbinah /kaikkiin tarvittaviin mikrotitrauslevyn syvennyksiin

For Research Use Only.

Not for use in diagnostic procedures.

CAUTION: Not for human or animal therapeutic or diagnostic use.

For in vitro use only.

For professional use only.

Read entire protocol before use.

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PACKAGE INSERT

Mediagnost Rat Adiponectin ELISA E091-R

- is suited for Adiponectin determination in **Rat Serum** and **Plasma** samples,
- is **fast**: incubation time a total of 2 hours and 30 minutes
- Single Standards with **0.25, 0.75, 1,5, 3, 6,5, and 10 ng/ml** native Rat Adiponectin are provided in the Kit
- 2 Control Sera are provided for quality control
- uses **high affinity monoclonal antibodies** against rat Adiponectin
- Microtiter plates are separately breakapart

Intended Use

Measurement of Adiponectin in Rat serum and plasma samples for research use.

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 rats usable in research and pre-clinical studies.

Even if the comparability of rats 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 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 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 $\mu\text{mol/kg}\cdot\text{min}$, total adiponectin as determined with the Mediagnost E091-R, 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 coronaritis [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, dived up in 12 stripes à 8 wells (separately breakapart), coated with anti-Rat Adiponectin antibody.
2)	CAL	Standards A-F , lyophilised, contain native Rat Adiponectin. Standard values are between 0.25 - 10 ng/ml (0.25, 0.75, 1.5, 3, 6.5 und 10 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 rat Serum and has to be reconstituted in 250 µl Dilution Buffer VP . The Adiponectin target value concentration and the respective range is given the vial label. 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-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)	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 H ₂ O ₂ Tetramethylbencidine.
8)	H ₂ SO 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
 Graduated cylinder for diluting Washing Buffer (WP)
 Distilled or deionized water for dilution of the Washing Buffer (WP), 950 mL
 Vortex-mixer
 Microtiter plate shaker (350 rpm)
 Microtiter plate washer (recommended)
 Micro plate reader ("ELISA-Reader") with filter for 450 and ≥ 590 nm.
 Polyethylene PE/Polypropylene PP tubes for dilution of samples

WARNINGS AND PRECAUTIONS

For research use only. For professional use only.

Do not use obvious damaged or microbial contaminated or spilled material.

Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements.

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.

Caution: This kit contains material of human and/or 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.

Following components contain < 0.01% 2-Methyl-4-isothiazolin-3-one solution as preservative : **A-F, AK, VP**

R34	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36/37	Wear suitable protective clothing and gloves
S45	In case of accident or if you feel unwell seek medical advice

Following components contain < 0.01%(w/w) 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-isothiazol-3-one as preservative: **A-F, AK, VP, WP**

R36/38	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water

Stop solution contains 0.2 M Sulfuric Acid (H₂SO₄)

R36/38	Irritating to eyes and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves.

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.

R20/21/R22	Harmful by inhalation, in contact with skin and if swallowed
R36/37/38	Irritating to eyes, respiratory system and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves

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-R 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 rats can be used in this assay.

Influence of Heparin (30IE/mL), EDTA (6,8mM) and NaCitrat (0,015M) on the measurement of Adiponectin has been investigated by recovery experiments. PBS was enriched with recombinant Rat 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

More than five freeze/thaw cycles are not possible.

Sample Preparation

Samples have to be diluted in Dilution Buffer (VP).

A sample dilution of 1:1500 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.

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

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 microtitre 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 microtitre plate washer, the respective instructions for 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 microtitre 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 2 months 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 . The labelled expiry is not influenced in case of proper storage.

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 be 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 in the first wells. Subsequently add 100 µl Standard or 100 µl of diluted Control Sera or diluted samples.
- 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 **3 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 **3 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 450nm (**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	0.25	0.75	1.5	3	6.5	10

- 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).
- 6) The Adiponectin concentration of the diluted sample or the diluted control sera KS1&2 in ng/ml (or µg/ml according the chosen unit for the standards) is calculated in this way, the Adiponectin concentration of the **undiluted sample** and of KS1 & KS2 is calculated **by multiplication** with the respective dilution factor.

Performance Characteristics

Standards

The Standards of the **ELISA E091-R** are prepared from **native Adiponectin** in concentrations of **0.25, 0.75, 1.5, 3, 6.5 and 10 ng/ml**. The native Adiponectin was calibrated against the recombinant Protein (Manufacturer: R&D Systems, Wiesbaden).

Sensitivity

The analytical sensitivity of the ELISA E091-R yields < 0,081 ng/ml (equal to 0,0081ng per well; as 2xSD of zero standard in 16fold determination).

Interassay Variability

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Mean [µg/mL]	1.62	7.06	7.97	7.22	11.23	8.55	7.24
SD	0.03	0.40	0.27	0.28	1.25	0.42	0.35
VC%	2.16	5.70	3.39	3.82	11.16	4.93	4.85
n	6	6	6	6	6	6	6

Intraassay Variability

	Sample 1	Sample 2
Mean [µg/mL]	12.702	5.709
SD	0.197	0.191
VC%	1.55	3.34
n	20	20

Linearity

Dilution	Sample 1 µg/mL	Dilution	Sample 2 ng/mL	Sample 3 µg/mL
1:600	5.2	1:1000	7.703	7.703
1:1200	5.091	1:2000	7.455	7.455
1:2400	5.286	1:4000	7.657	7.657
1:4800	5.117	1:8000	7.598	7.598
1:9600	5.008	1:16000	7.066	7.066

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SUMMARY – MEDIAGNOST RAT Adiponectin ELISA E091-R

Reagent preparation:	Reconstitution:	Dilution
Standards A-F	in 1 ml Dilution Buffer VP	
Control Sera KS1 & KS2	in 250 µl Dilution Buffer VP	1:1500 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 :1500		

Assay Procedure for Double Determination:

Pipette	Reagents	Position
100 µl	Dilution Buffer VP	A1/2
100 µl	Standard A (0.25 ng/ml)	B1/2
100 µl	Standard B (0.75 ng/ml)	C1/2
100 µl	Standard C (1.5 ng/ml)	D1/2
100 µl	Standard D (3 ng/ml)	E1/2
100 µl	Standard E (6.5 ng/ml)	F1/2
100 µl	Standard F (10 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		
3x 300 µl	Aspirate the contents of the wells and wash 3x with 300 µl Wash Buffer WP	each well
100 µl	Antibody-POD-Conjugate AK	each well
Cover the wells with the sealing tape.		
Incubation: 1 h at RT, 350 rpm		
3x 300 µl	Aspirate the contents of the wells and wash 3x 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-R



International Test description

CAL A-F	A -F	Rec in 1 ml VP	
Control	KS1 & KS2	Rec in 250 µl VP	1:1500 DILU VP
WASHBUF 20x	WP		1:20 DILU A. dest.

SPE	1:1500 DILU VP
°C 20-25 °C	

100 µl	VP	A1/2
100 µl	CAL A (0.25 ng/ml)	B1/2
100 µl	CAL B (0.75 ng/ml)	C1/2
100 µl	CAL C (1.5 ng/ml)	D1/2
100 µl	CAL D (3 ng/ml)	E1/2
100 µl	CAL E (6.5 ng/ml)	F1/2
100 µl	CAL F (10 ng/ml)	G1/2
100 µl	CONTROL KS1 1:1500 DILU VP	H1/2
100 µl	CONTROL KS2 1:1500 DILU VP	A3/4
100 µl	SPE 1:1500 DILU VP	
TAPE		

1 h 20-25 350 rpm

3x 300 µl	3x WASHBUF WP
100 µl	AbCONJ AK
TAPE	

1 h 20-25 350 rpm

3x 300 µl	3x WASHBUF WP
100 µl	SUBST TMB S

0.5 h 20-25

100 µl	H₂SO₄ SL
MEASURE	