Adiponectin

ELISA

Enzyme Immunoassay for Quantitative Determination of

human Adiponectin

English

For Research Use Only.
Not for use in diagnostic procedures.

+2-8°C

96 wells

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SYMBOLS/ SYMBOLE /SYMBOLES/ SIMBOLI/ SÍMBOLOS/ SÍMBOLOS/ SYMBOLEN/ SYMBOLER/ SYMBOLER/SYMBOLE/ SZIMBÓLUMOK/ SYMBOLY/ SYMBOLY/ СИМВОЛИ/ SÜMBOLID/ Σ΄ΥΜΒΟΛΑ/ SIMBOLURI/ SIMBOLI/ SYMBOLIT

DIN EN ISO 15223-1

	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 exspirácie/ Doba exspirace/ Срок на годност/ Aegumiskuupäev/ Ημερομηνία λήξης/ Data de expirare/ Rok uporabe/ Viimeinen käyttöpäivä
	Consider instructions for use/ Bitte Gebrauchsanweisung beachten/ Consultez la notice d'utilisation/ Consultare le istruzioni per l'uso/ Consulte las instrucciones de uso/ Respeitar as instruções de utilização/ A.u.b de gebruiksaanwijzing volgen/ Se brugsanvisningen/ Läs anvisningarna före användning/ Proszę przeczytać instrukcję obsługi/ Vegye figyelembe a használati utasításban foglaltakat/ Postupujte podľa pokynov na použitie/ Dodržujte návod k použití/ Моля, спазвайте инструкцията за употреба/ Palun järgige kasutusjuhendit/ Λάβετε υπόψη σας τις οδηγίες χρήσης/ Vă rugăm să respectaţi instrucţiunile de utilizare/ Upoštevajte navodila za uporabo!/ Lue käyttöohje huolellisesti!
	Lot-Batch Number/ Charge-Chargennummer/ Lot-Code du lot/ Lotto-Numero di lotto/ Lote-Código de lote/ Lote-Código do Lote/Lot-Partijnumme/ Lot-Batchkode/ Partisatskod/ Numer serii/ Tétel-sarzs szám/ Číslo šarže/ Číslo šarže/ Партиден номер/Partii number/ Παρτίδα-αριθμός παρτίδας/ Lot-număr lot/ Številka serije/ Erä
	Manufactured by/ Hergestellt von/ Fabriqué par/ Prodotto da/ Fabricado por/ Fabricado por/ Vervaardigd doo/ Fabrikation af/ Tillverkad av/ Wyprodukowane przez/ Gyártotta/ Vyrobené/ Vyrobeno v/ Производител/ Tootja/ Κατασκευάζεται από/ Produs de/ Proizvajalec/ Valmistaja
	Catalogue Number/ Bestellnummer/ Numéro de référence/ Numero di riferimento/ Número de referencia/ Número de Referência/ Referentienummer/ Referencenummer/ Beställningsnummer/ Numer katalogowy/ Rendelési szám/ Katalógové číslo/ Objednací číslo/ Каталожен номер/ Tellimisnumber/ Αρ. παραγγελίας/Număr comandă/ Številka naročila/ Viite tai tilausnumero
	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/ Säilitada 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/ Conţinut suficient pentru x teste/ Vsebina zadostuje za x preizkusov/ Sisältö riittää x testille
	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/ Pretrepať/ Protřepat/ Разклащане/ Raputada/ Ανακινήστε/ Vibrare/ Stresite/ Sekoita
	Reconstitute in/ Rekonstituieren in/ Reconstituer dans/ Ricostituire nel/ Reconstituir en/ Reconstituir em/ Reconstitueren in/ Rekonstituér i/ Rekonstituera/ Rekonstytuować w/ Helyreállítás/ Znovu pripraviť 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
	Miktrotiterplate/ Mikrotiterplatte/ plaque de microtitrage/ Piastra di microtitolazione/ Placa de microtitulación/ Placa de Microtitulação/ Microtiterplaat/ Mikrotiterplade/ mikrotiterplatta/ microtiterplaat/ Płytka microtiter/ Mikrotiter lap/ Mikrotitračná podložka/ Mikrotitrační podložka/ Микротитърна плака/ Mikrotiiterplaat/ Τρυβλίο μικροτιτλοδότησης/ Microplacă/ Mikrotitrska plošča/ Mikrotitrauslevy
	Antibody-Enzyme Conjugate/ Antikörper-Enzym Konjugat/ Anticorps conjuguée-conjugué enzymatique/ Coniugato di anticorpo-enzima/ Conjugado de anticuerpos-enzimas/ Conjugado Anticorpo-Enzima/ Antilichaam-- enzymconjugaat/ Antistoffer-enzym-konjugat/ Antikropps-enzymkonjugat (antikropp-enzym, konjugat)/ Koniugat antyciał-enzymów/ Antitest-enzim páros/ Protilátkový-enzymatický konjugát/ Protilátkový-enzymatický konjugát/ Антитяло-ензим конюгат/ Antikehad-ensüümi konjugaat/ Σύμπλοκο αντισώματος-ενζύμου/ Compuşi din anticorpi-enzime/ Antitelesa in konjugat encima/ Vasta-aine-entsymi konjugaatti
	Dilution Buffer/ Verdünnungspuffer/ Tampon de dilution/ Tampone di diluizione/ Tampón de dilución/ / Tampão de diluição / Verdunningsbuffer/ / Fortyndingsbuffer/ Utspädningsbuffert / Bufor rozcieńczający/ / Hígító puffer/ Riediaci pufor/ Ředicí pufr / Буфер за разреждане/ Lahjenduspuhver/ Ρυθμιστικό διάλυμα αραίωσης / Tampon de diluare/ Pufer za redčenje/ Laimennuspuskuri
	Dilute / Verdünnen / Diluer / Diluire / Diluir / Diluir / Verdunnen / Fortyndes / Späd / Rozcieńczanie / Hígítás / Riediť / Ředit / Разреждане / Lahjendada / Αραιώστε / Diluaţi / Razredčiti / Laimennetaan

CAL A-E	Calibrator X/ Kalibrator X/ calibrateur X/ calibratore X/ calibrador X/ calibrador X/ kalibrator X/ kalibrator X/ kalibrator X/ kalibrator X/ kalibrátor X/ kalibrátor X/ kalibrátor X/ калибратор X/ kalibraator X/ Βαθμονομητής X/ calibrator X/ kalibrator X/ kalibraattori X
CTR1 / CTR2	Control X/ Kontrolle X/ Contôle X/ controllo X/ control X/ Controle X/ controle X/ Kontrol X/ Kontroll X/ kontrolne X/ Ellenőrző X/ Kontrolné X/ Kontrolní X/ Контролен X/ Kontroll X/ ελέγχου X/ control X/ Kontrolni X/ Kontrolli X
WB	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 izpiralnega pufra/ Pesuliuositiiviste
WB 1:20	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
S	Substrate/ Substrat/ Substrat/ Substrato/ Substrato/ Substrato/ substraat/ Substrat/ Substrat/ Substrat/ Szubsztrátum/ Substrát/ Substrát/ Субстрат Substraat/ Υπόστρωμα/ Substrat/ Substrat/ Substraattiliuos
STP	Stop Solution/ Stopplösung/ Stop Solution/ Soluzione di stop/ Stop Solución/ Solução Stop/ stopoplossing/ Stopopløsning/ Stopplösning/ Stop roztwór/ Megállító oldat/ Roztok na ukončenie/ Roztok pro ukončení/ Стопиращ разтвор/ Stopp-lahus/ Διάλυμα διακοπής/ Soluţie 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 płytkę/ Tányér leragasztása/ Oblepiť podložku lepiacou páskou/ Olepit podložku lepicí páskou/ Плака с лента за запечатване/ Katta plaat isoleerkleeplindiga/ Κολλήστε το πλακίδιο με κολλητική ταινία/ Aoperiţi placa cu o bandă adezivă/ Prelepiti ploščo/ Peitä mikrotitrauslevy oheisella teipillä
MEASURE	Measure plate within 30 min at 450 nm (Referencefilter ≥590 nm)/ Ausmessung innerhalb von 30 min bei 450 nm (Referenzfilter ≥ 590 nm)/ Mesure lábsorbance en léspace de 30 min à 450 nm avec ≥590 nm longueur dónde 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 ≥ 590 nm)/ Binnen 30 minuten bij 450 nm meten (referentiefilter ≥ 590 nm)./ Mål plade i løbet af 30 min ved nm (referencefilter ≥590 nm)/ 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)/ Merať 30 minút pri 450 nm (Referenčných filtrov ≥590 nm)/ Měřit 30 minut při 450 nm (Referenční filtr ≥ 590 nm)/ Отчитане в рамките на 30 min при 450 nm (референтен филтър ≥ 590 nm)/ Mõõtmine 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 referinţă ≥ 590 nm)/ Izmerite ploščico v 30 min pri 450 nm (referenčni filter ≥590nm)/ Mittaa 30 minuutin aikana 450 nm:ssä (referenssi suodatin ≥ 590 nm)
Literature	Literature/ Literatur/ Bibliographie/ Letterario/ Bibliografía/ Literatura documentação/ 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/ instrucţiuni internaţionale 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 απαραίτητες κοιλότητες/ în toate cavităţile necesare/ v vseh zahtevanih vdolbinah/ kaikkiin tarvittaviin mikrotitrauslevyn syvennyyksiin

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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Mediagnost Adiponectin ELISA E09

Adiponectin ELISA E09	96 Determinations
Principle of the test	Enzyme-linked Immunoassay
Duration (incubation period)	1.75 h
Antibodies	monoclonal antibodies
Buffer	Ready for use and 20fold concentrate
Standard	5 single standards: 2 – 100 µg/L, native human Adiponectin
Assay Range	0.27 – 31000 µg/L
Control	2 controls freeze-dried
Sample	human serum / plasma
Required sample volume	10 µL
Sample dilution	1:310
Analytical sensitivity	ø ≤ 0.27 µg/L
Intra- / Interassay Variance	ø < 10 %

Intended Use
Measurement of human Adiponectin in human serum and plasma samples for research use!

Introduction
Adiponectin is a 30 kDa 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 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 [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

MTP	**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.
CAL A-E	**Calibrators**, lyophilised, contain native Adiponectin. Standard values are between **2-100 ng/mL** (2, 10, 30, 70 and 100 ng/mL) Adiponectin and have to be reconstituted in **750 µL (each) Dilution Buffer DIL**. 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 Calibrator frozen at -20°C. **Attention:** Calibrators should be thawed only once – where required please store aliquoted in adequate volumes.
DIL	**Dilution Buffer**, **125 mL**, ready for use, please use for the reconstitution of calibrators A-E, Controls CTR1 & CTR2 and for the sample dilution.
CTR1 / CTR2	**Control 1 & Control 2**, each **500 µL** lyophilised: Contains human Serum and has to be reconstituted in **500 µL Dilution Buffer DIL**. The Adiponectin target value concentration and the respective range are given on the certificate. The dilution of the Controls CTR1 & CTR2 should be according to the dilution of the respective samples, the target value concentration should be obtained by **multiplication** with the respective **dilution factor**.
DET	**Antibody-HRP-Conjugate**, **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.
WB	**Washing Buffer, 50 mL, 20 X concentrated** solution. **Washing Buffer WB** 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.
S	**Substrate, 12 mL,** ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised Tetramethylbencidine.
STP	**Stop Solution, 12 mL,** ready for use, 0.2 M sulphuric acid, Caution acid!
	Sealing tape for covering of the microtiter plate, 2 x, adhesive.

Materials Required but not provided

Precision pipettes Micropipettes and multichannel pipettes with disposable plastic tips
Distilled or deionized water for dilution of the Washing Buffer (WB)
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.

Not for internal use in humans and animals. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Mediagnost will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided. A Material Safety Data sheet is available on request.

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

Caution: This kit contains material of human and/or animal origin. Therefore, all components and patient´s 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.

Human Serum
Following components contain human serum: **Controls CTR1 and CTR2, and Calibrators CAL A-E**
Source human serum for the controls provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore, all components and patient´s specimens should be treated as potentially infectious.

Reagents CAL A-E, DET, DIL, WB
Contain as preservative a mixture of **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.

Substrate S
The TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbencidine (<0.05%)

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.

Stop Solution STP
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.

General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove 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: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

Method

The Mediagnost ELISA for Adiponectin E09 is a so-called Sandwich-Assay using two specific and high affine 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 heparin plasma levels are comparable. In EDTA- and Citrate Plasma-samples levels were found approx. 18% lower, because of the relatively high amount of anticoagulant. Adiponectin can also be measured in urine, breast milk and cellculture media by this testsystem.

The blood sample for serum preparation should be gained according to standardized venipucture procedure. The samples should be stored without anticoagulation reagents. 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 three freeze/thaw cycles are not possible.

Sample Preparation

Samples have to be diluted in **Dilution Buffer DIL**. The excellent linearity of this testsystem allows sample dilution of 1:200 to 1:1600.

We recommend a standard dilution of 1:310.

Suggestion for dilution protocol:
Dilute for example 300 µL Dilution Buffer **DIL** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 10 µL Serum- or Plasma (dilution: 1:31). Add **900 µL** Dilution Buffer **DIL** in another PE-/PP-tube and **100 µL** of the thoroughly mixed first dilution. After mixing, use **2×100 µL** from this **1:310** diluted sample in the assay.

Technical Notes

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 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.

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.

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.

Incubation at room temperature means: 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.

Calibrators and Controls

For the reconstitution of the lyophilised components (Calibrators A - E and Controls CTR1 &CTR2) the kit Dilution Buffer DIL 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 calibrators and controls can be stored for 1 month 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!

Assay Procedure
All determinations (Calibrators, Controls CTR1 & CTR2 and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

When performing the assay, the Calibrators, Controls 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-HRP-Conjugate **DET** as well as the following Substrate **S** should be added to the plate in the same order and in the same time interval as the samples. Stop Solution **STP** should be added to the plate in the same order as the Substrate Solution.

1) Add **100 µL Dilution Buffer DIL** in the first wells. Subsequently add **100 µL Calibrator** or **100 µL** of diluted **Controls** 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 WB** **WB 1:20** / 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-HRP-Conjugate DET** in each well.

5) Cover the wells with sealing tape and incubate the plate for **30 Minutes** at **room temperature** (shake at 350 rpm).

6) After incubation wash the wells **3 times** with **Washing Buffer WB** **WB 1:20** as described in step 3

7) Pipette **100 µL of the Substrate S** in each well.

8) Incubate the plate for **15 minutes in the dark at room temperature (20 - 25°C)**.

9) Stop the reaction by adding **100 µL of Stop Solution STP**.

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

Calculation of Results

Establishing the Calibration Curve

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

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

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

Calibrator	A	B	C	D	E
ng/mL	2	10	30	70	100
µg/mL	0.002	0.01	0.03	0.07	0.1

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 calibrator concentrations on the x-axis versus the mean value of the absorbance of the calibrators on the y-axis.

4) Recommendation: Calculation of the calibration curve should be done by using a computer program, because the curve is in general (without respective transformation) not ideally described by linear regression. **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 sample or the diluted Controls CTR1&CTR2 in ng/mL (or µg/mL according the chosen unit for the Calibrators) is calculated in this way, the Adiponectin concentration of the **undiluted sample** and of CTR1 & CTR2 is calculated **by multiplication** with the respective dilution factor.

The exemplary shown calibration curve in Fig.1 **cannot** be used for calculation of your test results. You have to establish a calibration curve for each test you conduct!
Exemplary calculation of the adiponectin concentration of a 1:310 diluted sample:

Measured extinction of your sample 0.39
Measured extinction of the blank 0.04
Your measurement programm will calculate the adiponectin concentration of the diluted sample automatically by using the difference of sample and blank for the calculation. You only have to determine the most suitable curve fit (here: polynomial 3rd degree).

In this exemplary case the following equation is solved by the programm to calculate the adiponectin concentration in the sample:

$$0.35 = 5 \times 10^{-7} x^3 - 0.0002x^2 + 0.0346x - 0.0166$$
$$11.13 = x$$

If the dilution factor (**1:310**) is taken into account the adiponectin concentration of the undiluted sample is

$$11.13 \times 310 = 3450.3 \text{ ng/mL} = 3.45 \text{ µg/mL}$$

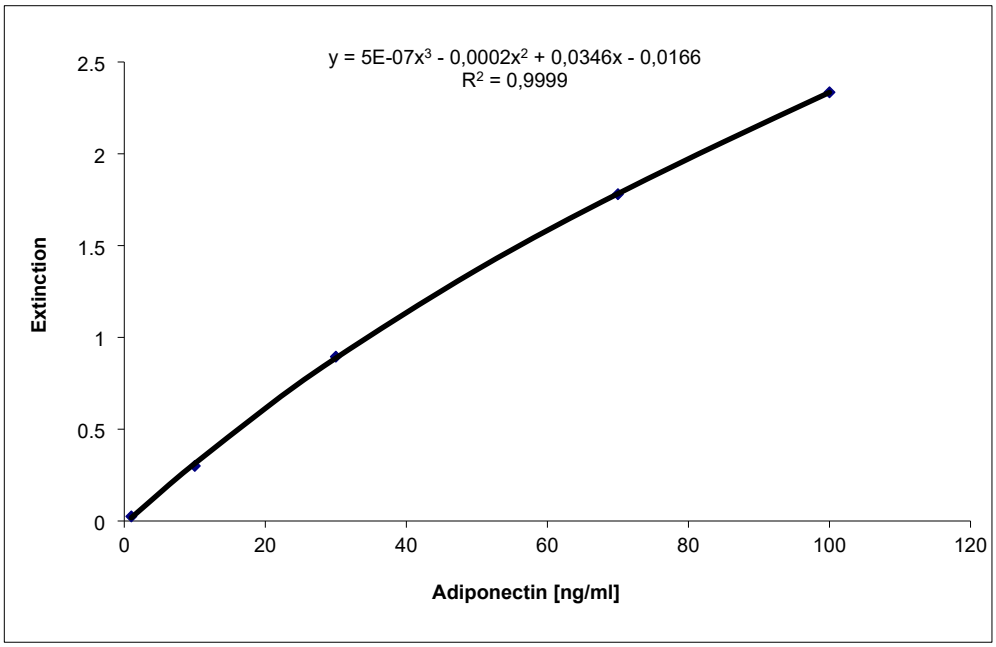

Figure 1. Exemplary Calibration Curve with a polynomial 3rd degree as curve fit.

Performance Characteristics

Calibrators

The calibrators of the ELISA E09 are prepared from **native Adiponectin** (Human Serum) in concentrations of 2, 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.

Sensitivity

The analytical sensitivity of the ELISA E09 was measured by the variability of the signal of the blank. Based on the twofold standard deviation of the blank the mean analytical sensitivity is < 0.27 ng/mL (Range 0.094 to 0.59 ng/mL).

Specificity

Adiponectin exists in different oligomeric forms: the high, medium and low molecular weight form. Different numbers of the adiponectin monomer aggregate specifically to form a complex. In Figure 2a the five different forms of human adiponectin are shown schematically. In parallel the results of a seize-exclusion chromatography of human serum measured with the Mediagnost E09 Adiponectin ELISA are shown.

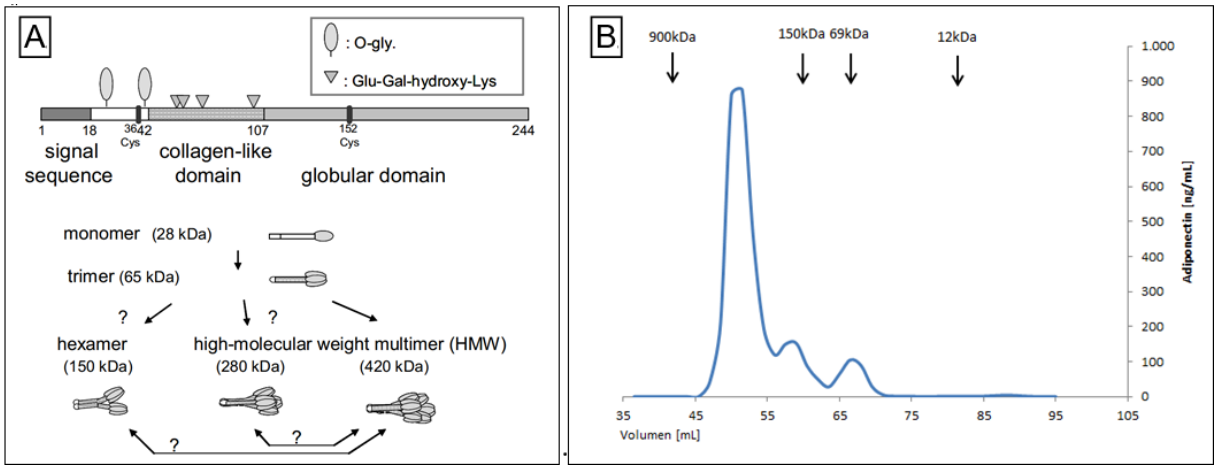

Figure 2 Adiponectin Structure (A) proteinaceous structure of human adiponectin including posttranslational modifications and multimeric forms (taken from Nakano et al[1]). (B) Results of a serum separation by seize-exclusion chromatography. The sample was fractionated and the adiponectin content of each fraction was measured by Mediagnost E09 Adiponectin, for comparison the corresponding seize in kDa is indicated by black arrows.

The results shown in Figure 2b clearly demonstrate that the Mediagnost E09 Adiponectin ELISA detects all forms of Adiponectin present in human serum: the trimer at 65 kDa, the hexamer at 150 kDa and the high molecular weight forms of >280 kDa. The Mediagnost E09 Adiponectin ELISA therefore measures total Adiponectin.

Serum of the cited species was diluted and used as sample in this assay system. No cross reactivity was detected:
Horse, Cow, Chicken, Rabbit, Dog, Guinea pig, Sheep, Mouse, Goat, Donkey, Rat, Cat
Whether this obvious non-reactivity is species specific should be assessed individually by each customer.

Interference

Interference of bilirubin and triglycerides was tested by adding different amounts of these substances to human serum containing adiponectin. For comparison the same amount of buffer without any substance was also added to the serum. Table 1 demonstrates that neither bilirubin nor triglycerides or haemoglobin exert any influence on the measurement of adiponectin in human serum.

Table 1 Interference. Serum samples were enriched with different amount of triglycerides, bilirubin or haemoglobin. The relative amount of adiponectin measured in comparison with native serum is shown here [%].

Triglyceride 100 mg/mL	Bilirubin 100 µg/mL	Hemoglobin 1 mg/mL	Hemoglobin 5 mg/mL
94	96	90	109
90	93	97	--
95	94	93	--

[1] Nakano Y, Tahima S, Yoshimi A, Akiyama H, Tsushima M, Tanioka T, Negoro T, Tomita M, Tobe T: A novel enyme-linked inmmunosorbent assay specific for high-molecular-weight adiponectin. J Lipid Res 2006, 47(7):1572-1582

Recovery

Trueness and traceability of the Mediagnsot Adiponectin ELISA E09 was evaluated by recovery of recombinant adiponectin in human serum. The recovery of recombinant Adiponectin yielded in a serum matrix on average 110%.

Table 2 Recovery of recombinant human Adiponectin in Serum. Recombinant Adiponectin was added in different amounts to human serum. The Adiponectin content of the so enriched samples was measured and recovery in comparison to enriched buffer calculated.

R&D recombinant Adiponectin lot 1022911	DIL	Serum	Recovery
ng/mL	ng/mL	ng/mL	%
0	0	0.00778	---
75	87.92	95.71	**109**
37.5	51.84	59.98	**116**
18.75	26.55	26.7	**101**
9.375	13.35	15.15	**113**

Precision

Intra-Assay Variance & Accuracy

Intra assay variance and accuracy is exemplarily shown with two samples. The adiponectin concentration of these samples was repeatedly measured in one assay.

Table 3 Intra-Assay Variation. Recombinant adiponectin was diluted in dilution buffer and the adiponectin concentration of the dilution was measured repeatedly within one assay.

	Determinations [n]	Mean value [µg/L]	Standard deviation [µg/L]	VC [%]	Target Value [µg/L]
Sample 1	8	7.108	0.22	3.14	6
Sample 2	8	107.96	3.97	3.67	100

In both samples the variability is less than 5% and the deviation from the target value is <20%.

Inter-Assay Variance

Serum samples where repeatedly measured in independent assays of different lots. On average the coefficient of variation was 7.5% (SD 1.6). The results of 5 samples are shown in table 4.

Table 4 Inter-Assay Variation

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean [µg/mL]	4.72	5.25	8.36	5.45	22.29
CV [%]	8.16	8.14	6.93	8.05	7.30
n	99	68	62	174	62

Linearity

Linearity of sample dilution was tested by serial dilution (1:100 – 1:4000) of human serum samples and recalculation of the adiponectin content in comparison to the mean adiponectin concentration of all dilutions (Table 5), no diluted sample showed a deviation of >30%.

Table 5 Linearity. Human serum sample were diluted in DIL and adiponectin content was recalculated. Measurements results are shown in [mg/L]. No deviation of the mean >30% was detected.

µg/mL	Mean	1:100	1:200	1:400	1:800	1:1000	1:2000	1:4000
Sample 1	5.76	6.53	6.331	5.764	5.49	6.067	6.114	4.056
Sample 2	11.53	10.93	12.107	11.395	11.454	11.567	12.884	10.362
Sample 3	12.07	13.57	12.853	12.03	11.974	11.338	11.548	11.169
Sample 4	4.89	4.659	4.886	4.384	4.425	5.851	5.13	n/a

Additionally, dilutions of 1:1500 to 1:96000 were evaluated with two samples. In Figure 3 the results are shown and demonstrate that in the tested samples no effect of dilution could be detected on measured adiponectin concentrations. The deviation of the target concentration of each dilution was less than 30% in all dilutions.

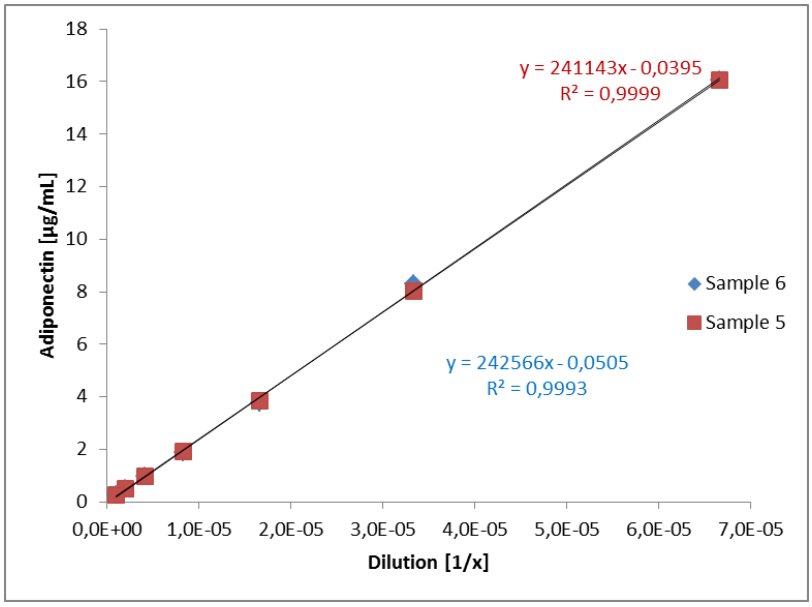

Figure 3 Linearity. Adiponectin concentration was measured in two human samples diluted 1:1500 to 1:96000.

Exemplary Values

The exemplary values for serum adiponectin, were measured with the Mediagnost ELISA E09 in healthy donors and analysed by Prof. Dr. J. Kratzsch, Department of Laboratory Medicine, University Hospital Leipzig, are given below (Tab. 6).

These data show significant correlation between Adiponectin-Serum values and age as well as gender 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.

Table 6a Exemplary values for **adults**, **gender specific** mean as well as median, 5. and 95. percentiles are given.

Sex	**number**	**Mean** [µg/mL]	**Median** [µg/mL]	**Standard Deviation**	**5th Percentile** [µg/mL]	**95th Percentile** [µg/mL]
Female	101	**10.2**	9.1	4.6	4.0	19.4
Male	125	**6.8**	6.1	4.1	2.0	13.9
total	226	**8.3**	7.5	4.6	2.4	19.3

Table 6b Exemplary values for **children**, **gender specific** mean as well as median, 5. and 95. percentiles are given.

Sex	**number**	**Mean** [µg/mL]	**Median** [µg/mL]	**Standard Deviation**	**5th Percentile** [µg/mL]	**95th Percentile** [µg/mL]
Female	131	**8.71**	8.18	4.32	3.05	15.6
Male	134	**8.97**	8.12	5.13	3.36	18.6
total	265	**8.84**	8.18	4.74	3.33	16.5

Table 6c Exemplary values for Adiponectin, **age specific** mean as well as median, 5. and 95. percentiles are given.

Age (in years)	**number**	**Mean** [µg/mL]	**Median** [µg/mL]	**5th Percentile** [µg/mL]	**95th Percentile** [µg/mL]
< 7.99	46	**12.82**	11.7	2.33	26.5
8 – 9.99	40	**8**	8.09	3.96	14.9
10-11.99	55	**8.02**	7.14	3.36	13.8
12 – 13.99	26	**8.21**	7.54	4.5	13.2
14 – 15.99	59	**8.12**	8.09	3.67	13.7
16 – 19.99	41	**7.97**	7.79	2.74	13.3
all	267	**8.88**	**8.18**	**3.33**	**16.7**
20 – 29.99	47	**6.72**	6.38	2.5	12.25
30 – 39.99	38	**7.38**	6.69	1.98	19.29
40 – 49.99	55	**8.42**	8.20	2.41	17.85
50 – 59.99	47	**9.61**	8.55	2.15	19.85
> 60	32	**9.52**	8.57	3.00	21.10
all	226	**8.33**	7.5	2.41	19.29

Table 6d Exemplary values for Adiponectin, **age** as well as **gender specific** mean and median, BMI and 25. and 75. percentiles are given.

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 Probands **AV=Average Value, BMI**=Body Mass Index (kg/m²) **SD**=Standard Deviation

Male			**Adiponectin (µg/mL):**			
Age (Years):	n:	**BMI: AV ± SD**	**AV ± SD:**	**Median:**	**Percentile: 25.- 75.**	**Min. – Max.:**
Newborn Cord blood	10		**27.80** ± 7.68	26.7	22.2-31.0	15.6-40.6
< 3.99	14	16.17 ± 1.81	**16.57** ± 6.55	14.3	11.6-21.2	5.8-40.3
4.0-7.99	12	15.69 ± 1.05	**11.24** ± 5.43	9.7	8.9-15.9	3.5-18.6
8.0-9.99	18	16.45 ± 1.76	**8.11** ± 2.93	7.6	6.2-9.1	5.00-15.4
10.0-11.99	21	18.34 ± 2.18	**8.43** ± 3.91	7.8	5.2-10.9	3.4-20.2
12.0-13.99	14	18.61 ± 2.11	**7.59** ± 2.86	7.1	6.0-10.3	2.4-12.2
14.0-15.99	32	19.86 ± 2.00	**7.53** ± 2.52	7.4	5.1-9.3	3.8-15.4
16.0-19,99	23	22.03 ± 2.42	**7.16** ± 3.53	6.9	4.2-9.6	2.0-13.9
20.0-29.99	23	23.43 ± 2.48	**5.44** ± 2.29	5.8	4.0-6.9	1.3-10.3
30.0-39.99	21	23.33 ± 2.72	**5.92** ± 4.60	4.4	2.7-6.7	1.9-20.6
40.0-49.99	22	23.79 ± 2.41	**6.13** ± 2.92	5.5	3.8-8.3	2.1-11.6
50.0-59.99	23	26.68 ± 2.77	**7.45** ± 4.50	6.7	5.0-8.8	1.4-19.6
>60.0	24	25.72 ± 2.12	**7.48** ± 3.92	7.6	4.6-9.2	3.0-21.1

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

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INTERNATIONAL TEST DESCRIPTION

International Test Description

CAL A-E	**Rec in 750 µL DIL**	**-**
CTR1	**Rec in 500 µL DIL**	**1:310 DIL**
CTR2	**Rec in 500 µL DIL**	**1:310 DIL**
WB 20x	**-**	**1:20 A. dest. ➔ WB 1:20**
SPE		**1:310 DIL**
-	**°C 20-25°C**	

100 µL	**DIL**	A1/A2
100 µL	**CAL A (2 ng/mL)**	B1/B2
100 µL	**CAL B (10 ng/mL)**	C1/C2
100 µL	**CAL C (30 ng/mL)**	D1/D2
100 µL	**CAL D (70 ng/mL)**	E1/E2
100 µL	**CAL E (100 ng/mL)**	F1/F2
100 µL	**CTR1 1:310 DIL**	G1/G2
100 µL	**CTR2 1:310 DIL**	H1/H2
100 µL	**SPE 1:310 DIL**	
TAPE		
1 h °C 20-25°C ↔ 350 rpm		
3x 300 µL	**3x WB 1:20**	
100 µL	**DET**	
TAPE		
0.5 h °C 20-25°C ↔ 350 rpm		
3x 300 µL	**3x WB 1:20**	
100 µL	**S**	
0.25 h °C 20-25°C		
STP		
MEASURE		

SUMMARY OF THE ASSAY PROCEDURE

Preparation of reagents		**Reconstitution:**	**Dilution**
CAL A-E	**Calibrators**	in **750 µL** Dilution Buffer **DIL**	-
CTR1 and CTR2	**Control 1 Control 2**	in **500 µL** Dilution Buffer **DIL**	**1:310** with **DIL**
WB	**Washing Buffer concentrate**	-	**1:20** with **Aqua dest.** ➔ **WB 1:20**
Sample dilution: with Dilution Buffer **DIL 1:310**			
Before assay procedure bring all reagents to room temperature **20-25°C**.			

Assay Procedure in Double Determination:

Pipette	**Reagents**	**Position**
100 µL	Dilution Buffer **DIL** (**Blank**)	A1/A2
100 µL	Calibrator **A** (**2 ng/mL**)	B1/B2
100 µL	Calibrator **B** (**10 ng/mL**)	C1/C2
100 µL	Calibrator **C** (**30 ng/mL**)	D1/D2
100 µL	Calibrator **D** (**70 ng/mL**)	E1/E2
100 µL	Calibrator **E** (**100 ng/mL**)	F1/F2
100 µL	Control **CTR1** (1:310 diluted)	G1/G2
100 µL	Control **CTR2** (1:310 diluted)	H1/H2
100 µL	Sample **SPE** (1:310 diluted)	in the rest of the wells according the requirements
Cover the wells with the sealing tape.		
Sample Incubation: **1 h at 20-25°C**, **350 rpm**		
3 x 300 µL	Aspirate the contents of the wells and **wash** 3 x with 300 µL each **WB 1:20 / well**	In each well
100 µL	Antibody-HRP-Conjugate **DET**	In each well
Cover the wells with the sealing tape.		
Incubation: 30 Minutes at 20-25°C, 350 rpm		
3 x 300 µL	Aspirate the contents of the wells and **wash** 3 x with 300 µL each Washing Buffer **WB 1:20/ well**	In each well
100 µL	Substrate Solution **S**	In each well
Incubation: 15 Minutes in the Dark at 20-25°C		
100 µL	Stop Solution **STP**	In each well
Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength.		