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User's Manual

Adiponectin ELISA

Enzymimmunoassay für die quantitative Bestimmung von humanem Adiponektin
Deutsch

Enzyme Immunoassay for quantitative Determination of human Adiponectin
English

IVD

CE

REF DEM-DEE009

Σ 96

Europäische Union / European Union*:
für in vitro Diagnostik / for in vitro diagnostics
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Symbols/ Symbole /Symboles/ Simboli/ Símbolos/ Símbolos/ symbolen/ Symboler/ Symboler/Symbole/ Szimbólumok/ Symboly/ Symboly/ Символи/ Sümbolid/ Σύμβολα/ Simboluri/ Simboli/ Symbolit

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

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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!

In vitro Diagnostic Medical Device (for in Vitro Diagnostic Use)/ in vitro Diagnostikum (zur In-vitro-Diagnostik)/ Dispositif médical de diagnostic in vitro (Pour usage diagnostique in vitro)/ Dispositivo medico per diagnostica in vitro (per uso diagnostico in vitro)/ Dispositivo médico de diagnóstico in vitro (para uso diagnóstico in vitro)/ Dispositivo Médico para Diagnóstico in vitro (Para Utilização de Diagnóstico in vitro)/ Medisch hulpmiddel voor diagnostiek in vitro (voor diagnostisch gebruik in vitro)/ Medicinsk udstyr til in vitro-diagnostik (Udelukkende til in vitro diagnostisk anvendelse)/ Medicinteknisk produkt avsedd för in vitro-diagnostik (för in vitro-diagnostiskt bruk)/ Wyrób medyczny do diagnostyki in vitro/ In vitro orvosdiagnosztikai termék (in vitro diagnosztikai használathoz)/ In vitro diagnostický zdravotnícky materiál (určené na diagnostiku „in vitro“)/ In vitro diagnostický zdravotnický materiál (určeno pro diagnostiku „in vitro“)/ Медицинско устройство за ин-витро диагностика (за ин-витро диагностика)/ in vitro diagnostikaseade (in vitro diagnostika tegemiseks)/ In vitro διαγνωστικό (για διάγνωση in vitro)/ Dispozitiv de diagnosticare in vitro (pentru diagnosticarea in-vitro)/ In vitro diagnostika (o in vitro diagnostiki)/ in vitro-diagnostiikkakäyttö

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 – 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

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/ Proteger da luz solar/ Niet aan zonlicht blootstellen/ Må ikke udsættes for sollys/ Utsätt inte för solljus/ Nie wystawiać na słońce/ Napfénytől távol tartandó/ Nevystavovať slnečnému svetlu/ Nevystavovat slunečnímu světlu/ Да се предпазва от слънчева светлина/ Kaitsta otsese päikesekiirguse eest/ Κρατήστε το μακριά από την ηλιακή ακτινοβολία/ Ţineţi 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/ Pretrepať/ Protřepat/ Разклащане/ Raputada/ Ανακινήστε/ Vibrare/ Stresite/ Sekoita

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

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

SPE		Sample/ Probe /Echantillon/ campione/ Muestra/ Amostra/ monster/ Prøve/ prov/ Próbka/ Minta/ Vzorka/ Vzorek/ Проба/ Proov/ Δείγμα/ Probă/Vzorec/Näyte
Ab CONJ	AK	Antibody and Enzyme Conjugate/ Antikörper und Enzym Konjugat/ anticorps conjuguée et conjugué enzymatique/ Coniugato di anticorpo ed enzima/ Conjugado de anticuerpos y enzimas/ Conjugado Anticorpo-Enzima/ antilichaam- en enzymconjugaat/ Antistoffer og enzym-konjugat/ antikropps- och enzymkonjugat (antikropp och enzym, konjugat)/ Koniugat antyciał i enzymów/ Antitest és enzim páros/ Protilátkový a enzymatický konjugát/ Protilátkový a enzymatický konjugát/ Антитяло и ензим конюгат/ Antikehad ja ensüümi konjugaat/ Σύμπλοκο αντισώματος-ενζύμου/ Compuşi din anticorpi şi enzime/ Antitelesa in konjugat encima/ Vasta-aine ja entsymi konjugaatti
DILU X	VP	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/ Rozcieńczanie w buforze X/ Hígítás X pufferben/ Riediť v pufri X/ Ředit v pufru X/ Разреждане в буфер X/ Lahjendada puhvris X/ Αραιώστε σε ρυθμιστικό διάλυμα X/ Diluaţi în tamponul X/ Razredčiti v pufru X/ laimennetaan x puskuriin
CAL X	A-E	Standard X /Standard X/ Etalon X/ Standard X/ Estándar X/ Standard X/ standaard X/ Standard X/ standard X/ Standard X/ Standard X/ Štandard X/ Standard X/ Стандарт X/ Standard X/ Πρότυπο X/ Standard X/ Standardni X/ Kalibraattori X
Control	KS1/ KS2	Control Serum / Kontrollserum/ Contôle sérique/ Siero di controllo/ Suero de control/ Soro de Controlo/ controleserum/ Kontrolserum/ Kontrollserum/ Serum kontrolne/ Ellenőrző szérum/ Kontrolné sérum/ Kontrolní sérum/ Контролен серум/ 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 izpiralnega pufra/ Pesuliuositiiviste
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/ Szubsztrátum/ Substrát/ Substrát/ Субстрат Substraat/ Υπόστρωμα/ Substrat/ Substrat/ Substraattiliuos
H_2SO_4	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 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 ≥590nm)/Ausmessung innerhalb von 30 min bei 450 nm (Referenzfilter ≥ 590 nm)./ Mesure lábsorbance en léspace de 30 min à450 nm avec ≥590nm 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 ≥590nm)/ Binnen 30 minuten bij 450 nm meten (referentiefilter ≥ 590 nm)./ Mål plade i løbet af 30 min ved 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)./ Merať 30 minút pri 450 nm/Měřit 30 minut při 450 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)
Literatur		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

Vor Gebrauch ist die gesamte Packungsbeilage zu lesen!

Read entire protocol before use!

Packungsbeilage
Deutsch

*please ask for package inserts in your national language

** please ask for special package insert

PACKAGE INSERT ENGLISH

Demeditec **Adiponectin ELISA DEE009 –**

- is suited for Adiponectin determination in **Serum** and **Plasma** samples,
- age- and sex specific **reference values** are available
- is extremely sensitive (less than **0.6 ng/ml**)
- is **fast**: incubation time a total of 1 hour and 45 minutes
- Single Standards with **2, 10, 30, 70, 100 ng/ml** human Adiponectin are provided in the Kit
- 2 Control Sera are provided for quality control purposes according GLP
- is calibrated with native **Adiponectin** and correlated to a commercialised radioimmunoassay
- uses **high affinity monoclonal antibodies** against human Adiponectin
- Microtiter plates are separately breakapart

Intended Use
Measurement of human Adiponectin in human serum and plasma samples.

Introduction

Adiponectin is a 30kDa protein which percentage in serum proteins is 0.01%. It is mainly synthesized by Adipocytes, but also muscle cells and hepatocytes have the ability to synthesize Adiponectin. Until now, IGF-I is the only known natural inductor of the synthesis. It consists of a Collagen-like N-terminal and a globular C-terminal domain [1]. In vivo Adiponectin appears with different oligomers. Beside the trimer and 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].
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 Demeditec DEE009, 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-human Adiponectin antibody.
2)	CAL	**Standards A-E**, 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 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-E, Control Sera KS1 & KS2 and for the serum dilution.
4)	Control	**Control Serum KS1 & KS2**, each **500 µl** lyophilised: Contains human Serum and has to be reconstituted in **500 µl Dilution Buffer VP**. The Adiponectin target value concentration and the respective range is given the QC data sheet. 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_2O_2 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
Microtiter plate shaker (350 rpm)
Microtiter plate washer (recommended)
Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm
Polyethylen PE/Polypropylen PP tubes for dilution of samples

WARNINGS AND PRECAUTIONS

For in-vitro diagnostic 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.

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 human and/or animal origin. Source human sera for the Control Sera provided in this kit were tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibodies. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.

Following components contain < 0.01% 2-Methyl-4-isothiazolin-3-one solution as preservative **A-E, 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-E, 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
S28.1	After contact with skin, wash immediately with plenty of water

Stop solution contains 0.2 M Sulfuric Acid (H_2SO_4)

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 Demeditec ELISA for Adiponectin DEE009 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 are applicable. In EDTA- and Citrate Plasma-samples levels were found approx. 18% lower. 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 five freeze/thaw cycles are not possible.

Sample Preparation
Samples have to be diluted in Dilution Buffer (VP). The excellent linearity of this testsystem allows sample dilution of 1:200 to 1:1600.

For clinical purposes we recommend a standard dilution of 1:310.

Suggestion for dilution protocol:
Dilute for example 300 µl Dilution Buffer VP in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 10 µl Serum- or Plasma (dilution: 1:31). Add **900 µl** Dilution Buffer **VP** in an other 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.

If you have the necessary technical equipment a one-step dilution of 1:301 is possible: Ad 5 µL to 1.5 mL dilution buffer VP.

Technical Notes

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

Reagents with different lot numbers cannot be mixed. The microtiter plate 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: 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 dynamicly 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 - E 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 pipette 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 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 **30 Minutes** 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 **15 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 E should be above 1.0.

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

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

Standard	**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 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 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 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.

The exemplary shown standard curve in Fig.1 **cannot** be used for calculation of your test results. You have to establish a standard 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

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

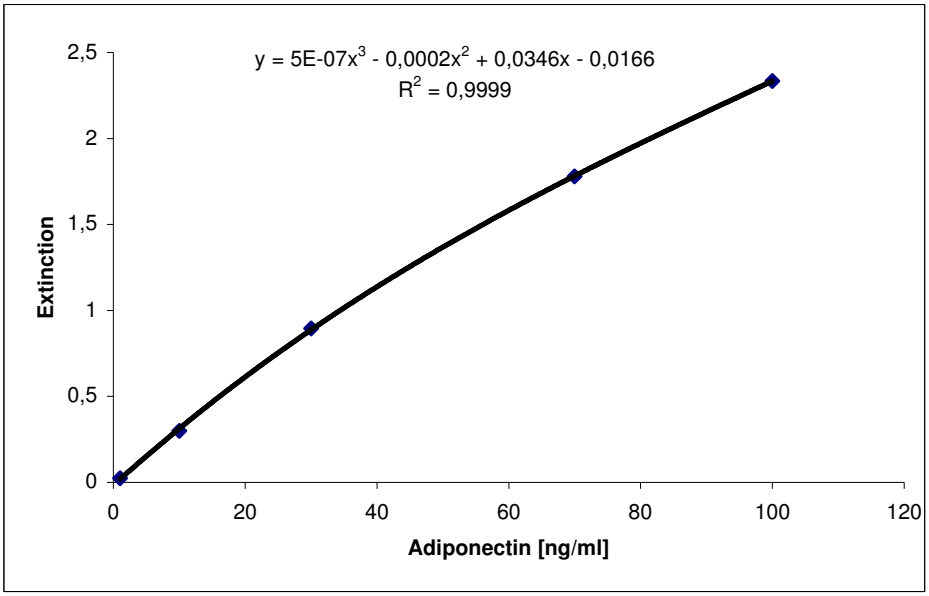

Fig. 1 Exemplary Standard Curve with a polynomial 3rd degree as curve fit.

Performance Characteristics

Standards

The Standards of the ELISA DEE009 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 DEE009 yields < 0.6 ng/ml (equal to < 0.06 ng per well; as 2xSD of zero standard in 16fold determination).

Specificity

Serum of the cited species was diluted (1:505) 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

Interference

Interference of physiological appearing substance with the adiponectin measurement was investigated. Serum samples have been enriched with different concentrations of possibly interfering substances and the amount of adiponectin was measured and compared with the adiponectin concentration in the same sample without any enrichment. In Table 1 the relative results are shown. None of the tested substances interfered significantly with adiponectin measurement.

Table 1: %-Recovery compared to non-enriched serum

	Triglyceride	**Bilirubin**	**Hemolysat**
%	100 mg/mL	100µg/mL	100µg/mL

Serum 1	95	97	90
Serum 2	90	93	97
Serum 3	95	94	93

Recovery

The recovery of recombinant Adiponectin yielded in a serum matrix on average 110%.

Table 2: Recovery of recombinant Adiponectin

Adiponectin [ng/mL]	9.38	18.75	37.5	75
% Recovery	109	116	101	113

Reproducibility and Precision

The inter- and intra assay coefficients of variability are below 6.7% and 4.7%, respectively. Exemplary determinations are shown in table 3 and 4.

Table 3: Inter-Assay-Variation

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

Table 4: Intra-Assay-Variation

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

Linearity

The Demeditec Adiponectin ELISA DEE009 is over a very wide range dilution authentic, the linearity of serum dilutions is over a very wide range excellent (s.Tab.5).

Table 5: Linearity

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

AV = Average Value , SD = Standard Deviation

Expected Reference Values

The expected values for serum adiponectin, which were determined with the Demeditec ELISA DEE009 in healthy donors and analysed by Prof. Dr. J. Kratzsch, Department of Laboratory Medicine, University Hospital Leipzig, are given below (Tab. 6). Several different statistical analyses were performed to adapt for certain individual demands. The best suited data can be chosen respectively for interpretation of the own measurements.

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: Expected values for **adults**, **gender specific** mean as well as median, 5. and 95. percentile 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: Expected values for **children**, **gender specific** mean as well as median, 5. and 95. percentile 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: Expected values for Adiponectin, **age specific** mean as well as median, 5. and 95. Percentile 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: Expected values for Adiponectin, **age** as well as **gender specific** mean and median, BMI and 5. and 95. percentile 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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KURZANLEITUNG – DEMEDITEC Adiponektin ELISA DEE009

Reagenz:	Rekonstitution:	Verdünnung:
Standards A-E	in 750 µl Verdünnungspuffer **VP**	
Kontrollseren KS1 & KS2	in 500 µl Verdünnungspuffer **VP**	**1:310 mit** Verdünnungspuffer **VP**
Waschpuffer WP		**1:20 mit Aqua. dest.** (z.B. den gesamten Flascheninhalt von (50 ml) im Standzylinder auf 1000 ml auffüllen)
Probenverdünnung: z.B. **1:310** (10 µl Serum werden mit 300 µl **Verdünnungspuffer VP** verdünnt, davon werden 100 µl in 900 µl Verdünnungspuffer verdünnt und von dieser 1:310 Verdünnung 100 µl/ Vertiefung einsetzen).		

Testdurchführung in Doppelbestimmungen:

Pipetieren	Reagenzien	Position
100 µl	Verdünnungspuffer **VP**	A1/2
100 µl	Standard **A** (**2 ng/ml**)	B1/2
100 µl	Standard **B** (**10 ng/ml**)	C1/2
100 µl	Standard **C** (**30 ng/ml**)	D1/2
100 µl	Standard **D** (**70 ng/ml**)	E1/2
100 µl	Standard **E** (**100 ng/ml**)	F1/2
100 µl	Kontrollserum **KS1**	G1/2
100 µl	Kontrollserum **KS2**	H1/2
100 µl	Probenverdünnung	nachfolgende Vertiefungen
Mit Klebefolie die Vertiefungen dicht abdecken.		
Inkubation: 1 h bei RT, 350 rpm		
3x 300 µl	Absaugen und die Platte **3x** mit je **300 µl** Waschpuffer **WP** / Vertiefung waschen.	In jede Vertiefung
100 µl	**Antikörper-POD-Konjugat AK**	In jede Vertiefung
Inkubation: 30 min bei RT, 350 rpm		
3x 300 µl	Absaugen und die Platte **3x** mit je **300 µl** Waschpuffer **WP** / Vertiefung waschen.	In jede Vertiefung
100 µl	Substratlösung **S**	In jede Vertiefung

Inkubation: 15 min im Dunklen bei RT

100 µl	Stopplösung **SL**	In jede Vertiefung
Ausmessung innerhalb von 30 min bei **450 nm** (Referenzfilter ≥590 nm).		

SUMMARY – DEMEDITEC Adiponectin ELISA DEE009

Reagent preparation:	Reconstitution:	Dilution
Standards A-E	in 750 µl Dilution Buffer **VP**	
Control Sera KS1 & KS2	in 500 µl Dilution Buffer **VP**	**1:310 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: Pipette for example 300 µl Dilution Buffer VP in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 10 µl Serum- or Plasma (dilution: 1:31). Add **900 µl** Dilution Buffer **VP** in an other 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.		

Assay Procedure for Double Determination:

Pipette	Reagents	Position
100 µl	Dilution Buffer **VP**	A1/2
100 µl	Standard **A** (**2 ng/ml**)	B1/2
100 µl	Standard **B** (**10 ng/ml**)	C1/2
100 µl	Standard **C** (**30 ng/ml**)	D1/2
100 µl	Standard **D** (**70 ng/ml**)	E1/2
100 µl	Standard **E** (**100 ng/ml**)	F1/2
100 µl	Control Serum **KS1**	G1/2
100 µl	Control Serum **KS2**	H1/2
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
Incubation: 30 min 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: 15 min in the **Dark** at **RT**		
100 µl	Stopping Solution **SL**	each well
Measure the absorbance within **30 min** at **450 nm** with **≥590 nm** as reference wavelength.		

RÉSUMÉ DU DOSAGE

Préparation des réactifs	Reconstitution:	Dilution
Etalons A-E	dans 750 µl de Tampon de Dilution **VP**	
Contrôle sérique KS1 & KS2	dans 500 µl de Tampon de Dilution **VP**	**1:310 avec** Tampon de Dilution **VP**
Tampon de lavage WP		**1:20** avec eau distilée. (ex. ajouter le contenu du flacon (50ml) dans une bouteille avec graduation et ajouter de l'eau distillée jusqu'à 1000 ml)
Diluer l´échantillon : Diluer par example 10 µl de sérum ou plasma dans 300 µl de WP, (dilution 1:31) dans des tubes PE/PP. Ajouter 100 µl de cette première dilution bien melangée à 900 µl de tampon de dilution dans un autre tube (dilution 1:310). Après mélange, utiliser 2 x100 µl de la dilution 1:310 d'échantillon pour le dosage.		

Procédure de dosage en doublets:

Distribuer:	Réactifs	Position
100 µl	Tampon de Dilution **VP**	A1/2
100 µl	Etalon **A** (**2 ng/ml**)	B1/2
100 µl	Etalon **B** (**10 ng/ml**)	C1/2
100 µl	Etalon **C** (**30 ng/ml**)	D1/2
100 µl	Etalon **D** (**70 ng/ml**)	E1/2
100 µl	Etalon **E** (**100 ng/ml**)	F1/2
100 µl	Contrôle sérique **KS1**	G1/2
100 µl	Contrôle sérique **KS2**	H1/2
100 µl	dilution d'échantillons	Les puits suivants
Recouvrir la microplaque avec une bande adhésive		
Incubation: 1 h à température ambiante, 350 tours par minute		
3x 300 µl	Aspirer le contenu des puits et laver 3 x avec 300 µl de tampon de lavage **WP**	Tous les puits
100 µl	Ajouter l'Anticorps-POD**-**Conjugate **AK**	Tous les puits
Incubation 30 min à température ambiante, 350 tours par minute		
3x 300 µl	Aspirer le contenu des puits et laver 3 x avec 300 µl de tampon de lavage **WP**	Tous les puits
100 µl	Ajouter la Solution de Substrat **S**	Tous les puits
Incubation: 15 min dans le noir à température ambiante		
100 µl	Ajouter la Solution d'arrêt **SL**	Tous les puits
Mesurer l'absorbance dans les 30 min à 450 nm avec ≥ 590 nm longueur d'onde de référence		

REF DEE0(**International Test description**

CAL **A-E**	**A -E**	**Rec in** 750 µl **VP**	
Control	**KS1 & KS2**	**Rec in** 500 µl **VP**	**1:310** **DILU** **VP**
WASHBUF **20x**	**WP**		**1:20** **DILU** **A. dest.**

SPE	**1:310** **DILU** **VP**
°C **20-25 °C**	

100 µl	**VP**	A1/2
100 µl	**CAL** **A** **A** **(2 ng/ml)**	B1/2
100 µl	**CAL** **B** **B** **(10 ng/ml)**	C1/2
100 µl	**CAL** **C** **C** **(30 ng/ml)**	D1/2
100 µl	**CAL** **D** **D** **(70 ng/ml)**	E1/2
100 µl	**CAL** **E** **E** **(100 ng/ml)**	F1/2
100 µl	**CONTROL** **KS1** **1:310** **DILU** **VP**	G1/2
100 µl	**CONTROL** **KS2** **1:310** **DILU** **VP**	H1/2
100 µl	**SPE** **1:310** **DILU** **VP**	
TAPE		

☐ **1 h** **°C** **20-25** ↔ **350 rpm**

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

☐ **0.5 h** **°C** **20-25** ↔ **350 rpm**

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

☐ **15 min** **°C** **20-25**

100 µl	**H_2SO_4** **SL**
MEASURE	