Chemerin

ELISA

Enzymeimmunoassay for quantitative Determination of

human Chemerin

English

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

Σ 96

REF **E102**

mediagnost®
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DIN EN ISO 15223-1

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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

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

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

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

STD 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/ Standardi 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/ 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

<u>For Research Use Only!</u>
<u>Not for use in diagnostic procedures!</u>
<u>CAUTION: Not for human or animal therapeutic or diagnostic use.</u>
<u>For in vitro use only!</u>
<u>For professional use only!</u>
<u>Read entire protocol before use!</u>

Mediagnost Chemerin ELISA E102

- for Research and professional use only
- is suited for Chemerin determination in **Serum** and **Plasma** samples
- is extremely **sensitive** (**5 pg/ml**) and thus allows measurements in cell culture media too and in specimens others than serum e.g. in cerebrospinal fluid, urine, breast milk and amniotic fluid
- is **fast**: incubation time a total of 2.5 hours
- Single Standards with **25, 100, 250, 400, 600 pg/ml** human Chemerin are provided in the kit
- 2 Control Sera are provided for quality control purposes according GLP
- is calibrated with **recombinant Chemerin**
- Microtiter plates are separately breakapart, tests can be adapted to individual requirements

INTENDED USE

Measurement of human Chemerin in human serum and plasma samples for research use only.

INTRODUCTION

Chemerin, also known as tazarotene-induced gene 2 (TIG2) or retinoic acid receptor responder 2 (RARRES2), is synthesized as precursor protein of 163 amino acids including a N-terminal signal peptide of 20 amino acids, which is chipped of enduring secretion. The inactive, circulation proform of Chemerin contains six cystein residues and thus three intramolecular disulfide bridges are suggested (1).
Prochemerin expression has been demonstrated for liver, lung, pituitary, lymph node, stomach and adipose tissue (1, 2). It has been detected in blood, ascitic fluids from ovary and liver cancer and synovial fluids from arthritic patients (1). Different receptors have been found in spleen, lymph node, small intestine, lung tissue as well as in macrophages and immature dendritic cells (1, 3).
Prochemerin is converted into its biologically active form by serine or cystein proteases, resulting in pro- or anti-inflammatory actions of the active protein, respectively. Proteolytic cleavage of Chemerin has been described for following serine proteases tryptase, plasmin, elastase, cathepsin G as well as for the cystein proteases cathepsin S and calpains (4-6).
The active protein is involved in innate and adaptive immune responses and for instance acts as a strong chemoattractant for immature dendritic cells and macrophages. It influences intracellular signaling by binding to its specific receptors. The C-terminal domain of Chemerin allows binding to the receptor ChemR23/CMKLR1 and elicits a pro-inflammatory stimulus by inducing Ca^{2+} influx and ERK1/2 activation. Further, the N-terminal domain of Chemerin binds to the CCRL2 receptor and allows presentation of the C-terminal domain to neighbor cells. In case of Prochemerin cleavage by cystein proteases the resulting peptides act inhibitory on the ChemR23/CMKLR1 receptors and thus have anti-inflammatory effects.
Recently, the relevance of Chemerin in adipogenesis and adipocyte metabolism has been discovered (2, 7). Goralski et al. demonstrated in mice that Chemerin as well as receptor (ChemR23) expression is present in adipocytes of visceral and subcutaneous adipose tissue. The expression and secretion increases enduring adipocyte differentiation. The active Chemerin of 16 kDa has been found in the conditioned media of adipocytes. Intracellular signaling networks in adipocytes are also influenced by Chemerin e.g. stimulation of MAPK p42/44 (ERK1/2) phosphorylation was demonstrated. Further, the authors also show a post-differentiational effect of Chemerin on gene expression in adipocytes.
Based on these results an influence of Chemerin on metabolic syndrome and insulin resistance was purposed. This studies reveal that Chemerin is more expressed by adipose tissue of obese humans and is able to impair insulin sensitivity of muscle cells and insulin mediated lipolysis and lipogenesis

in adipocytes (8, 9). It has been shown that Chemerin is associated with markers of inflammation and components of the metabolic syndrome (10, 11) as well as with renal function (12).

For the further investigation of Chemerin functions and its quality as biomarker in metabolic diseases reliable measurement of Chemerin in different body fluids is a prerequisite. Mediagnost offers a sensitive and reproducible test system for the quantitative measurement of Chemerin. Based on highly specific antibodies total Chemerin is measured.

MATERIALS PROVIDED

1)	MTP	**Microtiter plate**, ready for use, with 96 wells, dived up in 12 stripes à 8 wells (separately breakapart), coated with human Chemerin antibody.
2)	STD	**Standards A-E, lyophilised**, contain recombinant **Chemerin. Standard values are between 0.025 – 0.6 ng/ml** (25, 100, 250, 400 und 600 ng/ml) **Chemerin** and have to be reconstituted with **1 ml (each) Dilution Buffer VP.** Use 100 µl pro well in the assay.
3)	BUF	**Dilution buffer VP, 125 ml,** ready for use, after shaking. Please use this for the **reconstitution** of **Standards** and **Control Sera** and for the **dilution** of **Control Sera** and **Samples**.
4)	Control	**Control Sera KS1 and KS2**, **250 µl,** lyophilised, contain human Serum and should be **reconstituted in each 250 µl Dilution Buffer VP**. The Chemerin target values and the respective ranges are given on the certificate. The dilution should be according to the dilution of the respected samples. Use 100 µl pro well in the assay.
5)	Ab CONJ	**Antibody-HRP-Conjugate AK**, **12 ml,** ready for use, contains a mixture of biotinylated anti-human Chemerin Antibody and HRP (Horseradish Peroxidase)-labelled Streptavidin. Use 100 µl pro well in the assay.
6)	WASHBUF 20x	**Washing Buffer (WP), 50 ml, 20-fold 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 TMB	**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
Polyethylene PE/Polypropylene PP tubes for dilution of samples

WARNINGS AND PRECAUTIONS

For in-vitro use only. For professional use only.

Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.

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

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

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.

Human Serum

Following components contain human serum: **Control Sera KS1 / KS2, Standards A-E**

Source human serum for the control sera 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 AK, VP, WP, A-E

Contain as preservative **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 Solution (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.

Stopping Solution (SL)

The Stopping 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 Chemerin is a so-called Sandwich-Assay. It utilizes two specific and high affinity antibodies for this protein. The Chemerin in the sample binds to the immobilized first antibody on the microtiter plate. In the following step, the biotinylated and Streptavidin-Peroxidase conjugated second specific anti-Chemerin-Antibody binds in turn to the immobilised Chemerin. In the closing substrate reaction the turn of the colour will be high specific catalysed, quantitatively depending on the Chemerin-level of the samples.

SPECIMEN

Serum samples are suitable. Further neither EDTA (5.4 mmol/l), Sodium Citrate (10.6 mmol/l) nor Heparin (30 IU/ml) did interfere with Chemerin measurement.

Storage of the samples

Storage at RT max. 3 days

Storage at 4°C max. 6 days

Storage at –20°C max. 2 years

in tightly closable plastic tubes.

The measured values of serum and plasma samples did not show significant deviations up to 10 thaw/freezing cycles, values within the range of 92 to 104 % of the target value were found.

Sample Preparation

Samples have to be diluted in Dilution Buffer (VP).
In most determinations (serum or plasma samples, and no extreme values expected) a dilution **from 1:505 with Dilution Buffer VP** should be suitable. According to expected Chemerin levels the dilution with VP can be higher or lower. The excellent linearity of this test system allows sample dilution of 1:250 to 1:1000 (see table 6).

Chemerin concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatants (see table 1).

Suggestion for dilution protocol:
Pipette **1 ml Dilution Buffer VP** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **10 µl** Serum- or Plasma (dilution 1:101). Dilute in the second step **50 µl** of the **pre-diluted** sample with **200 µl Dilution Buffer VP** (dilution 1:505). After mixing use 100 µl per determination of this dilution in the assay.

TECHNICAL RECOMMENDATIONS

Reagents with different lot numbers cannot be mixed. All 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 limited to 4 weeks, if stored appropriately.

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming.

Incubation at room temperature means: Incubation at 20-25°C
The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must become adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variations and/or false values, excessive shaking may result in high optical densities and/or false values.

Proper washing is of basic importance for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.

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

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

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

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

Standards and Control

For the reconstitution of the lyophilised **Standards A - E Dilution Buffer VP** has to be used.

The lyophilised **Control Sera KS1 and KS2** must be **reconstituted** with the **Dilution Buffer VP**. The dilution should be according to the dilution of the respected samples 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 four weeks at –20°C. Repeated freeze/thaw cycles have to be avoided.

Washing Buffer

The required volume of Washing Buffer is prepared by 1:20 dilution of the provided 20-fold 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

Store the once unused microtiter strips and wells together with the desiccant in the tightly closed clip lock bag at 2-8°C and use in the frame provided. The labelled expiry is not influenced in case of proper storage.

Substrate Solution

The Substrate Solution (S), stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

ASSAY PROCEDURE

NOTES: All determinations (Standards, Control Sera 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). To avoid distortions due to differences in incubation times, the **Antibody-POD-Conjugate AK**, **the Substrate Solution S** as well as the **Stop Solution SL** should be added to the plate in the same order and in the same time interval each, respectively.

1) Add **100 µl Dilution Buffer VP** in wells A1/A2 (blank).
2) Pipette in positions B1/2 **100 µl of the Standard A** (25 pg/ml),
   pipette in positions C1/2 **100 µl of the Standard B** (100 pg/ml),
   pipette in positions D1/2 **100 µl of the Standard C** (250 pg/ml),
   pipette in positions E1/2 **100 µl of the Standard D** (400 pg/ml),
   pipette in positions F1/2 **100 µl of the Standard E** (600 pg/ml).
3) To control the correct accomplishment **100 µl** of the 1:505 (or in respective dilution rate of the sample) **in Dilution Buffer VP** diluted **Control Sera KS1 or KS2** can be pipetted in positions G1/2 and H1/2.
4) Pipette **100 µl** each of the **diluted sample** (e.g. dilute 1:505 with Dilution Buffer **VP)** in the rest of the wells, according to requirements.
5) Cover the wells with sealing tape and incubate the plate for **1 hour** at **room temperature** (shake at **350 rpm**). After incubation aspirate the contents of the wells and wash the **wells 5 times** with **300 µl Washing Buffer WP** / well.
6) Following the last washing step pipette **100 µl** of the **Antibody-POD-Conjugate AK** in each well.
7) **Cover the wells with the sealing tap**e and incubate **1 hour** at **room temperature** (shake at **350 rpm**)
8) After incubation wash the wells **5 times** with **Washing Buffer WP** as described in step 5.
9) Pipette **100 µl** of the **TMB-substrate** solution **S** in each well.
10) Incubate the plate for **30 minutes** in the dark at **room temperature**.
11) Stop the reaction by adding **100 µl** of **Stopping Solution SL** to all wells.
12) Measure the absorbance within **30 minutes** at **450 nm** (**reference filter**: ≥ 590 nm).

ESTABLISHING THE STANDARD CURVE

For the evaluation of the assay, it is preconditioned, that the absorbance values of the blank should be below 0.3, these of standard E should exceed 0.8.

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

The standards provided contain the following concentrations of Chemerin:

Standard	**A**	**B**	**C**	**D**	**E**
ng/ml	0.025	0.10	0.25	0.40	0.60
pg/ml	25	100	250	400	600

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 **Chemerin concentration** of the diluted sample or the diluted control sera in pg/ml (or ng/ml according the chosen unit for the standards) is calculated in this way, the Chemerin concentrations of the **undiluted samples** and of control sera are calculated **by multiplication with the respective dilution factor**.

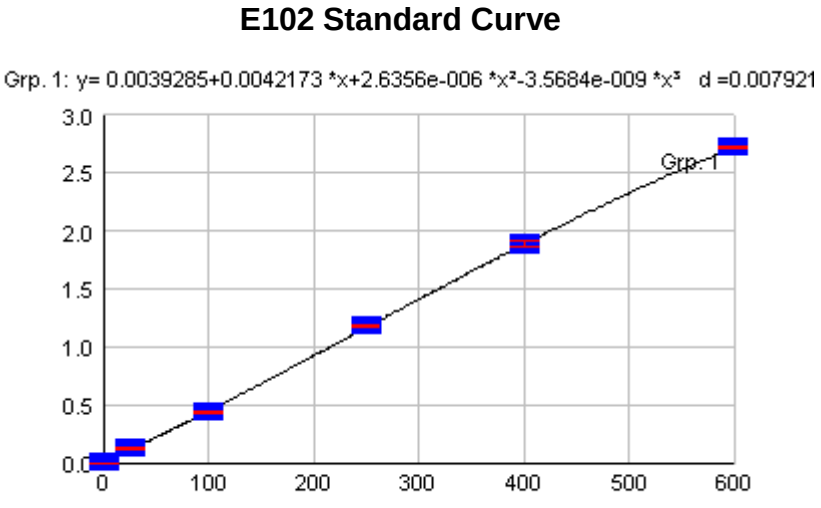

Fig. 1. Exemplary Standard Curve with a polynomial 3 as curve fit.

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 Chemerin concentration of a 1:505 diluted sample:

Measured extinction of your sample	0.56
Measured extinction of the blank	0.02

Your measurement program will calculate the Chemerin concentration of the diluted sample automatically by using the difference of sample (0.02) and blank for the calculation. You only have to determine the most suitable curve fit (here: polynomial 3 degree).

In this exemplary case, the following equation is solved by the program to calculate the Chemerin concentration in the sample:

$$0.54 = 0.0039285+0.0042173x+2.6356 \times 10^{-6}x^2-3.5684 \times 10^{-9}x^3$$

$$0.12 = x$$

If the dilution factor 1:505 is taken into account, the Chemerin concentration of the undiluted sample is $0.12 \times 505 = 60.65$ ng/ml

PERFORMANCE CHARACTERISTICS

Standards

The standards are prepared from recombinant human Chemerin (in concentrations of 25, 100, 250, 400 and 600 pg/ml (pico gram/ml, equal to 0.025 nano gram/ml-0.6 ng/ml).

Sensitivity

The **analytical sensitivity** of the assay yields **0.005 pg/ml** (5 pg/ml; as 2 x SD of zero standard in 19-fold determination).

Specificity

Commercially available sera from bovine, cat, chicken, dog, donkey, goat, guinea pig, horse, mouse, pig, rabbit, rat and sheep were diluted (1:10) and used as samples in this assay system and the signal intensity was measured. No cross reactivity was detected.

Recovery

The recovery of recombinant Chemerin in serum and plasma samples varied from 81 to 103%.

Matrix Effects

Table 1: Matrix effects: % Recovery of recombinant Chemerin in different body fluids.

Matrix effects						
Dilution [1:x]	**2**	**5**	**10**	**100**	**500**	**1000**
Saliva	35	52	70	-	-	-
Urine	92	92	91	-	-	-
Breast milk	90	88	86	-	-	-
Cell culture media	91	100	95	104	-	-
Cerebro-spinal fluid	> max.	> max.	> max.	96	92	94

- = not determided

Interference

Interference of physiological appearing substance with the Chemerin measurement was investigated. Serum samples have been enriched with different concentrations of possibly interfering substances and the amount of Chemerin was measured and compared with the Chemerin concentration in the same sample without any enrichment. In table 2 the relative results are shown. None of the tested substances interfered significantly with Chemerin measurement.

Table 2: %- Recovery compared to non-enriched serum.

	Triglycerides [100 mg/ml]	**Bilirubin** [200 µg/ml]	**Haemoglobin** [1 mg/ml]
%	107.6	98.6	108.5

Effects of coagulation inhibitors were investigating by adding indicated amounts of inhibitors to VP or PBS enriched with 125 pg/ml Chemerin. Relative amounts of Chemerin determined in inhibitor containing samples in comparison to inhibitor free samples are shown in table 3. None of the tested substances interfered significantly with Chemerin measurements.

Table 3: Effects of coagulation inhibitors.

		% Recovery
[3.8 g/l]	Citrate	103.4
[5.4 mmol/l]	EDTA	100.4
[30 IE/ml]	Heparin	103.0

Reproducibility and Precision

The inter and intra assay coefficients of variability are **below 5.16 and 2.17 %**, respectively. Exemplary determinations are shown in table 4 and table 5.

Table 4: Inter-Assay-Variation (results of 8 determinations, each).

	Mean value (ng/ml)	**Standard deviation**	**VC**
Probe 1	70.91	3.42	5.16
Probe 2	123.88	6.80	5.87
Probe 3	147.96	6.06	4.38

Table 5: Intra-Assay-Variation

	Number of	**Mean value**	**Standard deviation**	**VC**
Probe 1	20	84.33	1.71	2.02
Probe 2	20	142.33	3.93	2.76

Linearity

The Mediagnost Chemerin ELISA E102 is over a very wide range dilution authentic, the linearity of serum dilutions is over a dilution range of 1:250 to 1:1000 excellent (see table 6).

Table 6: Linearity of the Sample Dilution (characteristic results of three different sera).

Dilution	**Sample 1 [ng/ml]**	**Sample 2 [ng/ml]**	**Sample 3 [ng/ml]**
1:250	57.03	113.35	129.71
1:375	58.63	110.44	129.99
1:500	58.07	112.49	131.99
1:625	54.88	111.31	134.34
1:750	54.29	107.36	133.61
1:875	53.26	103.61	129.31
1:1000	53.51	101.59	126.43
AV / 1SD / VC%	**55.67 / 2.21 / 3.97**	**108.59 / 4.55 / 4.19**	**130.77 / 2.74 / 2.09**

AV= Average Value; SD = Standard Deviation, VC = Coefficient of Variation

EXEMPLARY VALUES

Concentrations of Chemerin in human sera of 40 healthy adult donors, at the age of 20 to 65 were determined with the Mediagnost ELISA E102. The concentrations of all samples varied from minimal 79.69 ng/ml to maximal 154.86 ng/ml (see table 7).

Table 7: Exemplary values in sera of healthy adults (measured values in ng/ml).

Gender	No. of samples	Average value [ng/ml]	Median [ng/ml]	Standard Deviation [ng/ml]	Min. – Max.: [ng/ml]
female	20	107.92	105.42	17.47	82.15-142.72
male	20	102.47	97.75	17.22	79.69-154.86
total	40	105.20	104.22	17.35	79.69-154.86

LITERATUR / LITERATURE

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SUMMARY – MEDIAGNOST CHEMERIN ELISA E102

Reconstitution / Dilution of Reagents		
Standards A-E	Reconstitution in **Dilution Buffer VP**	**1 ml each**
Control Serum KS1 & KS2	Reconstitution in **Dilution Buffer VP**	**250 µl each**
Washing Buffer WP	dilute in **A. dest.** (e.g. add the complete contents of the flask 50 ml into a graduated flask and fill with A.dest. to 1000 ml)	**1:20**
Sample Dilution + Control Sera KS1 & KS2: 1:505 in Dilution Buffer VP, **mix directly and use within max. 60 min.** Use **100 µl per determination**		
Before assay procedure bring all **reagents** to **room temperature**		

Proposal of Assay Procedure for Double Determination:

Pipette	Reagents	Well Positions
100 µl	Dilution Buffer **VP** as Blank	A1 and A2
100 µl	Standard **A** (**25 pg/ml**)	B1 and B2
100 µl	Standard **B** (**100 pg/ml**)	C1 and C2
100 µl	Standard **C** (**250 pg/ml**)	D1 and D2
100 µl	Standard **D** (**400 pg/ml**)	E1 and E2
100 µl	Standard **E** (**600 pg/ml**)	F1 and F2
100 µl	Control Serum **KS1**	G1 and G2
100 µl	Control Serum **KS2**	H1 and G2
100 µl	**Sample**	Pipette sample in the rest of the wells according to requirements
Cover the wells with the sealing tape		

Incubation: 1 h at RT, 350 rpm

5 x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP/well**	each well
100 µl	Antibody-POD-Conjugate **AK**	each well

Incubation: 1 h at RT, 350 rpm

5 x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP/well**	each well
100 µl	Substrate Solution **S**	each well

Incubation: 30 min in the dark at RT

100 µl	Stop Solution **SL**	each well
Measure the absorbance within 30 min at **450 nm** (≥590 nm Reference)		

REF E102 **International Test Description**

STD A-E	**A -E**	**Rec in** 1 ml **BUF VP**	
Control	**KS1&KS2**	**Rec in** 250 µl **BUF VP**	
WASHBUF 20x	**WP**		**1:20 DILU A. dest.**

Control	**1:505 DILU BUF VP**
SPE	**1:505 DILU BUF VP**
°C 20-25 °C	

100 µl	**BUF VP**	A1/2
100 µl	**STD A (25 pg/ml)**	B1/2
100 µl	**STD B (100 pg/ml)**	C1/2
100 µl	**STD C (250 pg/ml)**	D1/2
100 µl	**STD D (400 ng/ml)**	E1/2
100 µl	**STD E (600 pg/ml)**	F1/2
100 µl	**CONTROL KS1 1:505 DILU BUF VP**	G1/2
100 µl	**CONTROL KS2 1:505 DILU BUF VP**	H1/2
100 µl	**SPE 1:505 DILU BUF VP**	
TAPE		

À 1 h °C 20-25 ↔ 350 rpm

5 x 300 µl	**5x WASHBUF WP**
100 µl	**Ab CONJ AK**
TAPE	

À 1 h °C 20-25 ↔ 350 rpm

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

À 0.5 h °C 20-25

100 µl	**H_2SO_4 SL**
MEASURE	