Resistin

ELISA

Enzyme Immunoassay for quantitative Determination of

human Resistin

English

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

mediagnost®
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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

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 respectați instrucțiunile de utilizare/ Upoštevajte navodila za uporabo!/ Lue käyttöohje huolellisesti!

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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

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Store at between/ Lagerung bei zwischen/ Conserver à entre/ Conservare a tra/ Conservar a temp. Entre/ Armazenar entre/ Bewaar bij tussen/ Opbevares mellem/ Förvaras vid/ Przechowywać w/ Tárolási tartomány/ Skladujte v rozsahu / Skladujte v rozmezí/ Температурно ограничение/ 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/ Conținut suficient pentru x teste/ Vsebina zadostuje za x preizkusov/ Sisältö riittää x testille

Keep away from sunlight/ Nicht dem Sonnenlicht aussetzen/ Conserver à l´abri de la lumière/ Conservare al riparo della luce solare/ No exponer a la luz solar/ 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/ Țineți departe de lumina soarelui/ Ne izpostavljajte sončni svetlobi/ suojaa auringonvalolta

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

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

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

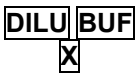
Miktrotiterplate/ Mikrotiterplatte/ plaque de microtitrage/ Piastra di microtitolazione/ Placa de microtitulación/ Placa de Microtitulação/ microtiterplaat/ Mikrotiterplade/ mikrotiterplatta/ microtiterplaat/ Płytka microtiter/ Mikrotiter lap/ Mikrotitračná podložka/ Mikrotitrační podložka/ Микротитърна 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)/

EK 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



PP/ 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/ Diluați în tamponul X/ Razredčiti v pufru X/ laimennetaan x puskuriin

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
BUF X	PP/ VP	Buffer / puffer/ Tampon / Tampone / Tampón /Tampão / buffer/ buffer/ buffert/ Bufor / puffer/ pufer/ pufr/ буфер/ puhver/ ρυθμιστικό διάλυμα / Tampon /pufer/ puskuriliuos
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/ Soluție de oprire/ Stop raztopina/ Pysäytysliuos
TAPE		Cover Plate with sealing tape /Platte abkleben/ Recouvrir la microplaque avec bande adhésive/ Coprire la piastra con nastro adesivo/ Cubrir la placa con una cinta adhesiva/ Cobrir a Placa com fita adesiva/ plaatje met tape afdekken/ Afdækningsplade med tape/ maskera platta/ Odkleić 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/ Aoperiți 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 referință ≥ 590 nm)./ Izmerite ploščico v 30 min pri 450 nm (referenčni filter ≥590nm) / Mittaa 30 minuutin aikana 450 nm:ssä (referenssi suodatin ≥ 590 nm)
Literatur		Literature/ Literatur/ Bibliographie/ Letterario/ 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/ instrucțiuni internaționale pentru testare/ mednarodna navodila za preizkus/ Kansainvälinen käyttöohje
End		in all required wells/ in allen benötigten Vertiefungen/ dans tous les godets requis/ in tutti i pozzetti richiesti/ en todos los pozos requeridos/ em todos os tubos necessários/ in alle nodige putjes/ i alle nødvendige brønde/ i alla nödvändiga brunnar/ we wszystkich potrzebnych wgłębieniach/ minden szükséges forrásban/ vo všetkých potrebných miestach/ ve všech potřebných místech/ във всички необходими ямки/ kõigis vajalikes süvendites/ σε όλες τις απαραίτητες κοιλότητες/ în toate cavitățile 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!

Mediagnost Resistin ELISA E50

- **for research use only!**
- is suited for Resistin determination in **Serum** and **Plasma** samples
- is extremely **sensitive** (**12 pg/ml ≅ 1.2 pg per well**) and, thus allows measurements in cell culture media too and in specimens others than serum e.g. in Cerebrospinal fluid, Amnion fluid, Saliva, Urine, Breast milk
- is **fast**: incubation time a total of 4 hours
- Single Standards with **20, 100, 300, 600,1000 pg/ml** human Resistin are provided in the Kit
- 2 Control Sera for Quality Control
- is calibrated with **recombinant Resistin**
- Microtiter plates are separately breakapart, tests can be adapted to individual requirements

INTENDED USE

Measurement of human resistin in human serum and plasma sample for research use.

INTRODUCTION

Resistin, a cysteine-rich protein of 11.3 kDa (1), was firstly found in mice (2) and constitutes together with RELMα, RELMβ and RELMγ the protein family of resistin-like molecules (RELM).

In humans, resistin and RELMβ (1) but no other proteins of the RELM family were found. The human form of resistin shows a homology of 53% to the murine protein (4). It has 11 cysteine-residues, is synthesized as a propetide of 108 amino acids and secreted as a dimer, build by a disulfide bridge of cysteine residues (22). Beside this intermolecular disulfide bridge, 5 additional intramolecular ones exist (5,6).

Appearance of multi- and oligomer formation was proved by size exclusion chromatography. Thereby it was shown, that oligomer formation is SDS-insensitive but can be inhibited by β-mercaptoethanol and is therefore likely to be caused by disulfide bridges (1). Further on, the resistin structure seems to be dependent on its concentration, as circular dichroism analysis shows a concentration dependent shift of α-helical to β-sheet structure (1).

Resistin expression was demonstrated in white adipose tissue (10), pituitary (11) and pancreatic islets (12) of mice as well as in brown adipose tissue of rats. In humans, resistin expression in adipocytes can be detected but only at a very low level. But in vitro, resistin expression of non-adipocytes in fatty tissue was shown (13). Human resistin gene is also expressed in pancreatic islets (12), pre-adipocytes (14 marcophages (15) and bone marrow (39). So, resistin is of relevance for inflammation processes as well as for lipid metabolism.

Most investigation refers to the mouse model. Here, the existence of trimeric and hexameric resistin in serum was demonstrated (7). In comparison to adiponectin biology it is highly probable that different resistin oligomers have different biologic function (8, 9).

In mice, a correlation between adiposity, insulin resistance and resistin expression was found empirically. In humans, respective study results are not clear – several studies show an association of resistin serum concentration and adiposity or insulin resistance (17, 25-31). But others failed in confirming these results (14, 16-24). Therefore, there is requirement for valid and reproducible determination of resistin serum concentration.

Relevance of resistin in other physiologic processes than energy metabolism was investigated by several different approaches. Experiments with endothelial cells gave interesting results. Here, resistin was shown to enhance expression of VCAM-1 and ICAM-1 (33, 34). By this way, resistin is potentially able to influence endothelial inflammation (35, 36) and, thereby atherosclerosis.

These results were confirmed by experiments in mice, where endothelin-1 was shown to regulate resistin secretion (37, 38).

In recent research human resistin was shown to increase pre-adipocyte proliferation and lipolysis of mature adipocytes (38). By the way of modulating MAPK-signalling pathways resistin exerts crucial influence on energy metabolism.

This ELISA-kit enables the user to determine the exact concentration of Resistin in human serum/plasma as well as other body fluids and thereby assists investigation of Resistin biology.

REAGENTS PROVIDED

1)	MTP	**Microtiter plate**, ready for use, with 96 wells, dived up in 12 stripes à 8 wells (separately breakapart), coated with human Resistin antibody.
2)	CAL	**Standards A-E, lyophilised, contain** recombinant **Resistin. Standard values are between 0.02 - 1 ng/ml** (20, 100, 300, 600 und 1000 pg/ml) **Resistin and have to be reconstituted** with **750 µl (each) Sample Buffer PP.** Attention: Please use only Sample Buffer PP for this dilution, because only this assures, that the Standards and the respective samples subsequently will incubate under identical conditions in the same special buffer!
3)	BUF	**Sample Buffer PP, 120 ml,** ready for use, please use for the reconstitution of the Standards A – E and for the dilution of samples and Control Sera KS1 and KS2.
4)	BUF	**Dilution buffer VP, 25 ml,** ready for use, please use this for the **reconstitution** of **Control Sera KS1/KS2** and for the **dilution** of **Antibody Conjugate AK** and **Enzyme Conjugate EK**.
5)	Control	**Control Sera KS1 and KS2**, lyophilised: Contain human Serum and have to be reconstituted with **250 µl (each) Dilution buffer VP.** The Resistin target values and the respective ranges are given on the vial label. The dilutions of the **Control Sera KS1 and KS2** in **Sample Buffer PP** should be according to the dilution of the respective samples.
6)	Ab	**Antibody Conjugate AK, 120 µl, 100fold concentrated solution,** contains biotinylated anti-Resistin antibody, please dilute before use 1:100 in **Dilution buffer VP**: e.g., add 100 µl **Antibody Conjugate AK** to 10 ml **Dilution Buffer VP**, mix and use 100 µl/well of this dilution in the assay.
7)	CONJ	**Enzyme Conjugate EK, 120 µl, 100fold concentrated solution,** contains HRP (Horseradish peroxidase)-labelled Streptavidin, please dilute before use 1:100 in Dilution Buffer VP: e.g. add 100 µl Enzyme conjugate EK to 10 ml Dilution buffer VP, mix and use 100 µl/well of this dilution in the assay.
8)	WASHBUF 20x	**Washing Buffer (WP), 50 ml, 20fold 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 at 2-8°C, dilute only according to requirements.
9)	SUBST	**Substrate (S), 12 ml**, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2 Tetramethylbencidine.
10)	H_2SO_4	**Stopping Solution (SL), 12 ml,** ready for use, 0.2 M sulphuric acid, Caution acid!
11)		Sealing tape for covering of the microtiter plate, 3 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes and multichannel pipettes with disposable plastic tips
Graduated cylinder for diluting Washing Buffer (WP)
Distilled or deionized water for dilution of the Washing Buffer (WP), 950 mL
Vortex-mixer
Microtiter plate Shaker (350 rpm)
Microtiter plate washer (recommended)
Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm
Polyethylene PE/Polypropylene PP tubes for dilution of samples

WARNINGS AND PRECAUTIONS

For research and 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. **Temperature will affect the absorbance** readings of the assay. However, values for the samples will not be affected.

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

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

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

Caution: This kit contains material of human and/or animal origin. Source human serum for the Control Sera provided in this kit was 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 specimens should be treated as potentially infectious.

2-Methyl-4-Isothiazolin-3-one
Following components contain < 0.01% **2-Methyl-4-isothiazolin-3-one** solution as preservative **A-E, AK, EK, VP, PP**

< 0.01% 2-Methyl-4-isothiazolin-3-one Solution

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

5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-Isothiazol-3-one
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, EK, VP, WP, PP**

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 S	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 enzyme immunoassay for Resistin E50 is a so-called Sandwich-Assay. It utilizes a specific high affinity polyclonal rabbit antiserum coated on the wells of a microtiter plate. The Resistin in the samples binds quantitatively to the immobilized antiserum. In the following step, the biotinylated antiserum binds in turn to Resistin. After washing, Streptavidin-Peroxidase-Enzyme conjugate will be added, which will bind highly specific to the biotin of the antiserum and will catalyse in the closing substrate reaction the turn of the colour, quantitatively depending on the Resistin level of the samples.

SPECIMEN

Serum as well plasma samples are suitable (significant deviation of Resistin levels in corresponding serum-, Heparin-, EDTA-, Citrate-plasma-Samples were not found). Haemolytic samples appear to show falsely high Resistin levels, using such samples should be checked out critically. Common cell culture medium, saliva, breast milk and urine were found to be suitable specimens too.
By means of the special sample buffer an external sample preparation prior to the assay is not required (see below).

The blood sample for serum preparation should be gained according to standardized venipuncture procedure. The samples should be stored without anticoagulation reagents. Haemolytic 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 min. 2 years

in tightly closable plastic tubes.

The storage of samples, over a period of 2 years at -20°C, showed no effect on the measured value.

Freeze/thaw cycles of samples should be minimized.

Sample Preparation

Samples have to be diluted in Sample Buffer (PP). The excellent linearity of this test system allows sample dilution of 1:5 to 1:400.

In most determinations (serum or plasma samples, and no extreme values expected) a dilution **from 1:10 to 1:50 with Sample Buffer PP** should be suitable. According to expected Resistin levels the dilution with PP can be higher or lower. Because the sample buffer **PP** is special composed for the correct determination of Resistin, the dilution should be **at least 1:5**!
Resistin concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatants.

We recommend a standard dilution of 1:21.

Suggestion for dilution protocol:
Pipette 300 µl Sample Buffer PP in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 15 µl Serum- or Plasma (dilution1:21). After mixing use 2 x 100 µl of this dilution in the assay.

ASSAY PREPARATION AND 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 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: **20-25°C**

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

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

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

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

When using an automatic microtitre plate washer, the respective instructions 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 microtitre plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non fuzzy absorbent tissue.

Standards and Controls

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

The lyophilised **Control Sera KS1 and KS2** must be reconstituted with the **Dilution Buffer VP**. The dilution of the **Control Sera in Sample Buffer PP** should be according 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 **4 weeks at –20°C**. Repeated freeze/thaw cycles have to be avoided.

Antibody and Enzyme Conjugate

Use the Dilution Buffer **VP** for the dilution of the Antibody Conjugate **AK** and Enzyme Conjugate **EK** 100fold concentrates. The diluted solutions are only limited stable at 2-8°C and should be prepared daily fresh.

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!

Microtiterplate

After initial opening, store the unused strips and microtiter wells together with the desiccant in the tightly closed clip lock bag at 2-8°C and use in the frame provided.

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 pipetted as fast as possible (e.g., <15 minutes). To avoid distortions due to differences in incubation times, **Antibody-Conjugate AK** and the **Enzyme Conjugate EK** 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 Sample Buffer PP** in wells A1/A2 (blank) and
2) Pipette in positions B1/2 **100 µl of the Standard A** (0.02 ng/ml),
   pipette in positions C1/2 **100 µl of the Standard B** (0.1 ng/ml),
   pipette in positions D1/2 **100 µl of the Standard C** (0.3 ng/ml),
   pipette in positions E1/2 **100 µl of the Standard D** (0.6 ng/ml),

pipette in positions F1/2 **100 µl of the Standard E** (1 ng/ml).

3) To control the correct accomplishment **100 µl** of the 1:21 (or in respective dilution rate of the sample) **in Sample Buffer PP** diluted **Control Sera KS1 and KS2** can be pipetted in positions G1/2 and H1/2.

4) Pipette **100 µl** each of the **diluted sample** (e.g. dilute 1:21 with Sample Buffer **PP)** in the rest of the wells, according to requirements.

5) Cover the wells with sealing tape and incubate the plate for **2 hours** 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 1:100 with **Dilution Buffer VP** diluted **Antibody Conjugate AK** in each well. Cover the wells with the sealing tape and incubate **1 hour** at room temperature (shake at 350 rpm).

7) After incubation wash the wells 5 times with **Washing Buffer WP** as described in step 5).

8) Following the last washing step, pipette **100 µl** of the 1:100 with Dilution Buffer VP diluted **Enzyme Conjugate EK** in each well. Cover the wells with the sealing tape and incubate for **30 minutes** at room temperature (shake at 350 rpm).

9) After incubation wash the wells 5 times with Washing Buffer **WP** as described in the step 5.

10) Pipette **100 µl** of the **TMB-substrate** solution **S** in each well.

11) Incubate the plate for **30 minutes** in the dark at **room temperature**.

12) Stop the reaction by adding **100 µl** of **Stopping Solution SL** to all wells.

13) Measure the absorbance within **30 minutes** at **450 nm** (reference filter: ≥ 590nm).

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 be above 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 Resistin:

Standard	**A**	**B**	**C**	**D**	**E**
ng/ml	0.02	0.10	0.30	0.60	1.00
pg/ml	20	100	300	600	1000

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

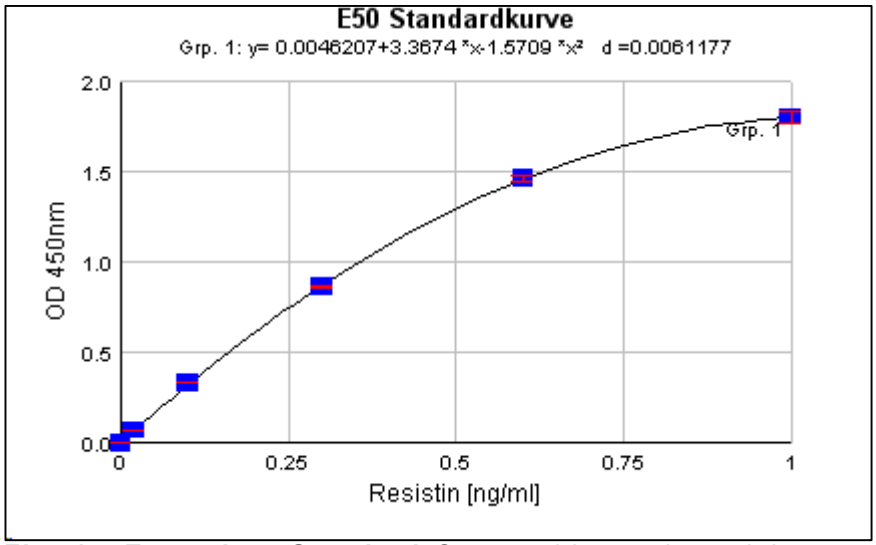


Fig. 1. Exemplary Standard Curve with a polynomial 2nd degree as curve fit.

The exemplary standard curve shown 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 Resistin concentration of a 1:21 diluted sample:

Measured extinction of your sample	0.85
Measured extinction of the blank	0.05

Your measurement program will calculate the Resistin 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 2nd degree).

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

$$y = 0.0046207 + 3.3674\,x - 1.5709x^2$$
$$0.2686 = x$$

if the dilution factor (1:21) is taken into account the Resistin concentration of the undiluted sample is

$0.2686 \times 21 = 5{,}64$ ng/mL = 0.00564 µg/mL

PERFORMANCE CHARACTERISTICS

Standards

The standards are prepared from recombinant human Resistin (19.5 kDa, 2 x 92 amino acids, expressed in E. coli) in concentrations of 20, 100, 300, 600 and 1000 pg/ml (pico Gramm / ml, equal to 0.02 ng/ml-1 ng/ml).

Sensitivity

The **analytical sensitivity** of the assay yields **0.012 ng/ml** (12 pg/ml; as 2x SD of zero standard in 15fold 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.

Interference

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

Table 1: Interference: Three serum samples where enriched with indicated amount of the potentially interfering substance and measured. Shown is % of Resistin of the native, non enriched serum sample

	Triglyceride 100 mg/ml	**Bilirubin 100 µg/ml**	**Haemolysate 1000 µg/ml**
Serum 1	101	93	94
Serum 2	115	99	99
Serum 3	104	103	147

Table 2: Effects of coagulation inhibitors were investigating by adding indicated amounts of inhibitors to PP enriched with 0.3 ng/ml Resistin. Relative amounts of Resistin measured in inhibitor containing samples in comparison to 0.3 ng/ml Resistin containing Sample Buffer (PP) are shown.

		% of Resistin in PP	
		Mean (n=3)	**SD**
3.8 g/l	Citrate	94	7.67
0.0068 mol/l	EDTA	93	4.96
30,000 IE/l	Heparin	96	4.89

Reproducibility and Precision

The inter- and intra assay coefficients of variability are below than 6.8% and 5%, respectively. Exemplary determinations are shown in table 3 and table 4.

Table 3: 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**

Table 4: Inter-Assay-Variation (results of 11 determinations, each)

	Mean value (ng/ml)	**Standard deviation (ng/ml)**	**VK (%)**
Sample 1	2.70	0.16	5.94
Sample 2	4.20	0.28	6.77
Sample 3	5.80	0.28	4.79

Recovery and Linearity

The Mediagnost Resistin ELISA E50 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: Recovery and linearity of the Sample Dilution (characteristic results of two different sera)

Dilution	**Sample 1** (native 5.5 ng/ml)		**Sample 2** (native 2.25 ng/ml)	
	plus 5 ng/ml	Recovery (%)	plus 12.25 ng/ml	Recovery (%)
1:50	9.71	92.5	14.99	103.4
1:100	10.60	101.0	13.64	94.1
1:200	10.44	99.4	14.10	97.2
1:400	10.32	98.3	14.33	98.8

Different human sera were spiked with recombinant human Resistin in varying concentrations (e.g. in Table 6). The recovery of Resistin yielded on average 98 % of the theoretically expected amount.

Table 6: Samples were enriched with 0.3 ng/ml Resistin and measured in comparison to non enriched sample. Relative recovery of added Resistin is shown.

Matrix	**Dilution**	**% Recovery**
Cerebrospinal fluid	1:2	129
Cerebrospinal fluid	1:10	93
Cerebrospinal fluid	1:40	103
Amnion fluid	1:10	85
Amnion fluid	1:40	91
Saliva	1:10	99
Saliva	1:21	86
Urine	1:10	79
Urine	1:21	85
Breast milk	1:2	97
Breast milk	1:10	58
Breast milk	1:21	63
Cell culture supernatant	1:2	100

EVALUATION OF RESULTS

Table 7: The exemplary values for Resistin were determined with the Mediagnost ELISA E50 in healthy probands and analysed by Prof. Dr. J. Kratzsch, Institute for Laboratory Medicine, University of Leipzig.

Female				**Resistin (ng/ml):**		
Age (Years):	**n:**	**AV Age:**	**AV BMI:**	**AV ± SD:**	**25.- 75. Percentile:**	**Min. – Max.:**
18 - 30	96	23.0	23.1	**7.2 ±** 2.6	5.4 – 8.8	3.1 – 14.7
31 - 40	63	36.5	24.3	**8.1 ±** 2.3	6.4 – 9.6	3.6 – 13.1
41 - 50	67	44.9	24.8	**7.3 ±** 2.5	5.7 – 8.1	4.0 – 16.1
51 - 60	29	54.7	25.0	**7.2 ±** 2.6	5.4 – 8.5	4.0 – 15.5
61 - 65	9	62.7	25.2	**6.6 ±** 1.1	6.0 – 6.7	5.4 – 9.3
Male				**Resistin (ng/ml):**		
Age (Years):	**n:**	**AV Age:**	**AV BMI:**	**AV ± SD:**	**25.- 75. Percentile:**	**Min. – Max.:**
18 - 30	107	23.9	24.1	**6.4 ±** 1,8	5.0 – 7.6	2.5 – 13.1
31 - 40	59	35.9	25.0	**6.7 ±** 3,2	4.8 – 7.4	3.8 – 26.9
41 - 50	66	45.0	25.2	**6.5 ±** 2,8	4.5 – 7.4	2.4 – 16.7
51 - 60	36	54.8	26.4	**6.1 ±** 2,1	4.7 – 7.2	3.2 – 13.3
61 - 68	20	63.2	25.6	**7.2 ±** 1,8	6.0 – 8.2	4.5 – 11.2

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

Table 8: Grouping of the expected values

Sex	**Number**	**Mean [ng/ml]**	**Standard deviation**	**2.5. Percentile**	**9.5. Percentile**
Male	288	6.48	2.44	3.32	11.68
Female	264	7.41	2.47	3.68	13.60
Total	552	6.93	2.49	3.58	13.12

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SUMMARY – MEDIAGNOST RESISTIN ELISA E50

Reagent preparation:	**Reconstitution:**	**Dilution:**
Standards A – E	in **750 µl** Sample Buffer **PP**	
Control Serum KS1	in **250 µl** Dilution Buffer **VP**	**1:21** with Sample Buffer **PP**
Control Serum KS2	in **250 µl** Dilution Buffer **VP**	**1:21** with Sample Buffer **PP**
Antibody Conjugate AK		**1:100** with Dilution Buffer **VP**
Enzyme Conjugate EK		**1:100** 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: **1:21** (e.g. 15 µl Serum with 300 µl Sample Buffer **PP)**		

Assay Procedure for Double Determination

Pipette	**Reagents**	**Position**
100 µl	Sample Puffer **PP** (blank value)	A1/2
100 µl	Standard **A** (0.02 ng/ml)	B1/2
100 µl	Standard **B** (0.1 ng/ml)	C1/2
100 µl	Standard **C** (0.3 ng/ml)	D1/2
100 µl	Standard **D** (0.6 ng/ml)	E1/2
100 µl	Standard **E** (1.0 ng/ml)	F1/2
100 µl	Control Serum **KS1** (diluted)	G1/2
100 µl	Control Serum **KS2** (diluted)	H1/2
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
Incubation: 2 h at **RT, 350 rpm**		
5x 300 µl	Aspirate the contents of the wells and wash 5x with **300 µl** Wash Buffer **WP**	each well
100 µl	**1:100** diluted Antibody Conjugate **AK**	each well
Incubation: 1 h at **RT, 350 rpm**		
5x 300 µl	Aspirate the contents of the wells and wash 5x with **300 µl** Wash Buffer **WP**	each well
100 µl	**1:100** diluted Enzyme Conjugate **EK**	each well
Incubation: 30 min at **RT, 350 rpm**		
5x 300 µl	Aspirate the contents of the wells and wash 5x with **300 µl** Wash Buffer **WP**	each well
100 µl	Substrate Solution **S**	each well
Incubation: 30 min in the dark at **RT**		
100 µl	Stop Solution **SL**	each well
Measure the absorbance within **30 min** at **450 nm** with **≥590 nm** as reference wavelength		

REF E50  **International Test description**

CAL A-E	**A –E**	**Rec in** 750 µl **PP**	
Control	**KS1/KS2**	**Rec in** 250 µl **VP**	**1:21 DILU BUF PP**
AB	**AK**		**1:100 DILU BUF VP**
CONJ	**EK**		**1:100 DILU BUF VP**
WASHBUF 20x	**WP**		**1:20 DILU A. dest.**
SPE			**1:21 DILU BUF PP**
°C 20-28 °C			

100 µl	**BUF PP**	A1/2
100 µl	**CAL A (0.02 ng/ml)**	B1/2
100 µl	**CAL B (0.1 ng/ml)**	C1/2
100 µl	**CAL C (0.3 ng/ml)**	D1/2
100 µl	**CAL D (0.6 ng/ml)**	E1/2
100 µl	**CAL E (1 ng/ml)**	F1/2
100 µl	**CONTROL KS1 1:21 DILU PP**	G1/2
100 µl	**CONTROL KS2 1:21 DILU PP**	H1/2
100 µl	**SPE 1:21 DILU PP**	
TAPE		

2 h °C 20-25 ↔ 350 rpm

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

1 h °C 20-25 ↔ 350 rpm

5x 300 µl	**5x WASHBUF WP**
100 µl	**CONJ EK**
TAPE	

0.5 h °C 20-25 ↔ 350 rpm

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

30 min °C 20-25 

100 µl	**H_2SO_4 SL**
MEASURE	