Progranulin

ELISA

Enzymeimmunoassay for quantitative Determination of

human Progranulin

English

For Research Use Only.
Not for use in diagnostic procedures

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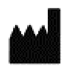
Symbols/ Symbole /Symboles/ Simboli/ Símbolos/ Símbolos/ Symbolen/ Symboler/ Symboler/ Symbole/ Szimbólumok/ Symboly/ Symboly/ Символи/ Sümbolid/ Σύμβολα/ Simboluri/ Simboli/ Symbolit

DIN EN ISO 15223-1

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

Consider instructions for use/ Bitte Gebrauchsanweisung beachten/ Consultez la notice d'utilisation/ Consultare le istruzioni per l'uso/ Consulte las instrucciones de uso/ Respeitar as instruções de utilização/ A.u.b de gebruiksaanwijzing volgen/ Se brugsanvisningen/ Läs anvisningarna före användning/ Proszę przeczytać instrukcję obsługi/ Vegye figyelembe a használati utasításban foglaltakat/ Postupujte podľa pokynov na použitie/ Dodržujte návod k použití/ Моля, спазвайте инструкцията за употреба/ Palun järgige kasutusjuhendit,/ Λάβετε υπόψη σας τις οδηγίες χρήσης/ Vă rugăm să respectaţi instrucţiunile de utilizare/ Upoštevajte navodila za uporabo!/ Lue käyttöohje huolellisesti!

Lot-Batch Number/ Charge-Chargennummer/ Lot-Code du lot/ Lotto-Numero di lotto/ Lote-Código de lote/ Lote-Código do Lote/Lot-Partijnumme/ Lot-Batchkode/ Partisatskod/ Numer serii/ Tétel-sarzs szám/ Číslo šarže/ Číslo šarže/ Партиден номер/Partii – partii number/ Παρτίδα-αριθμός παρτίδας/ Lot-număr lot/ Številka serije/ erä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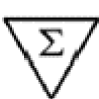
Catalogue Number/ Bestellnummer/ Numéro de référence/Numero di riferimento/ Número de referencia/ Número de Referência/ Referentienummer/ Referencenummer /Beställningsnummer/ Numer katalogowy/ Rendelési szám/Katalógové číslo/ Objednací číslo/Каталожен номер/Tellimisnumber/ Αρ. παραγγελίας/Număr comandă/ 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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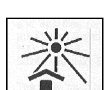
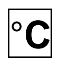
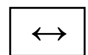

Keep away from sunlight/ Nicht dem Sonnenlicht aussetzen/ Conserver à l´abri de la lumière/ Conservare al riparo della luce solare/ No exponer a la luz solar/ Proteger da luz solar/ Niet aan zonlicht blootstellen/ Må ikke udsættes for sollys/ Utsätt inte för solljus/ Nie wystawiać na słońce/ Napfénytől 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

CONJ
AK
EK
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

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

Symbol	Code	Description
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

For in vitro use only!
For Research Use Only.

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

Read entire protocol before use!

Symbols/ Symbole /Symboles/ Simboli/ Símbolos/ Símbolos/ Symbolen/ Symboler/ Symboler/ Symbole/ Szimbólumok/ Symboly/ Symboly/ Символи/ Sümbolid/ Σύμβολα/ Simboluri/ Simboli/ Symbolit 2

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Mediagnost Progranulin ELISA E103

- For research and professional use only!
- is suited for Progranulin determination in **Serum** and **Plasma** samples
- is extremely **sensitive** (**18 pg/ml**) 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 2 hours
- Single Standards with **75, 250, 750, 1500, 2500 pg/ml** human Progranulin are provided in the Kit
- 2 Control Sera are provided for quality control purposes according GLP
- is calibrated with **recombinant Progranulin**
- Microtiter plates are separately breakapart, tests can be adapted to individual requirements

INTENDED USE

Measurement of human Progranulin in human serum and plasma sample for research use!

INTRODUCTION

Progranulin is also known as Granulin Epithelin Precursor, Proepithelin or Acrogranin. It is a 68.5 kDa protein, consisting of 593 amino acids (inclusive Signalpeptid), which appears in vivo in strongly glycosylated form and therefore has a size of approximately 90 kDa (1).
Progranulin has seven conserved domains, which are separated by linker sequences. By means of proteolytic cleavage, catalyzed by serine proteases like e.g. elastase, 6-25 kDa large fragments result, that are called Granulines or Epithelines. Progranulin is expressed and secreted in particular in strongly proliferating tissues such as adenoid tissue, spleen, skin epithelium, gastrointestinal mucous membranes, haematopoietic cells and in tumor cells. Until now no specific receptors, which would obtain the effect of Progranulin or the Granulines are known (2, 3).
Progranulin seems to be a factor, which affects the wound healing positively. In case of skin lesions the expression is increased in ceratinocytes, in macrophages and in neutrophile cells. Progranulin affects the wound healing indirectly by activation of macrophages and stimulation of angiogenesis in the damaged tissue (4). The physiological effects of Progranulin and Granulines are oppositional. Progranulin can restrain TNFα mediated pro-inflammatory processes. On the other hand the Granulines seem to stimulate the secretion of pro-inflammatory cytokines. The influence of Progranulin on inflammatory processes could be shown also in arteriosclerotic plaques. Here Progranulin is expressed by smooth muscle cells and affects the migration of monocytes and smooth muscle cells (5). In the central nervous system Progranulin is expressed in microglia and neurons (in neocortical and hippocampal pyramid cells as well as in purkinje cells in the cerebellum).
On mRNA level a clear increase of Progranulin expression could be shown during infections or injuries of the CNS, for example in mucopolysaccharidosis type I and IIIB, in viral inflammations of CNS, in amyotrophic lateral sclerosis and in Alzheimer's disease. Beyond that Progranulin seems to be of relevance in the development of sex specific differences during pre- and postnatal development and also for the neural plasticity in adults (6).

Progranulin and Frontotemporal Dementia (FTD)
5-10 % of all dementias are of the frontotemporal form. A mutation in the gene for Progranulin (PGRN) could be shown in 5-10 % of the humans suffering FTD (2). Nearly all pathological mutations lead to a premature transcription interruption and to rapid degradation of the mutated mRNA. This results in a PGRN haploinsufficiency with clearly decreased Progranulin concentrations in serum. Due to these results several studies were accomplished, in order to clarify the suitability of Progranulin as marker for the PRGN dependent frontotemporal dementia (7, 8).

Progranulin and Adiposity
Inflammatory processes are often increased in case of adiposity and type 2 diabetes, which is reflected by e.g. in the increase of the C-reactive Protein and pro-inflammatory cytokines e.g. IL-6. Youn et al. compared different groups of obese humans and have shown that the plasma concentration of Progranulin is significantly (1.4-fold) increased in type 2 diabetics compared to glucose-tolerant humans. The authors refer in particular to the positive correlation of the Progranulin concentration to the volume of the visceral adipose tissue. On the other hand no difference between slim and subcutaneous obese humans has been detected in this study. For this reason the increase of the Progranulin concentration may reflect the body distribution of adipose tissue and thus represent a biomarker for visceral adipose tissue (9).
The Mediagnost Progranulin ELISA E103 is based on monoclonal antibodies, which detect with high specificity only Progranulin and not the single Granulines. Thus, a tool is available for the further investigation and validation of Progranulin as a biomarker for the visceral adipose tissue.

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 Progranulin antibody.
2)	STD	**Standards A-E, lyophilised**, contain recombinant **Progranulin. Standard values are between 0.075 – 2.5 ng/ml** (75, 250, 750, 1500 und 2500 pg/ml) **Progranulin** and have to be reconstituted with **1 ml (each) Dilution Buffer VP.** Use 50 µl pro well in the assay.
3)	BUF	**Dilution buffer VP, 50 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 Progranulin target values and the respective ranges are given on the vial labels. The dilution should be according to the dilution of the respected samples. Use **50 µl** pro well in the assay.
5)	Ab	**Antibody Conjugate AK, 6 ml**, ready for use, contains the biotinylated anti-Progranulin antibody. Use 50 µl for each well in the assay.
6)	CONJ	**Enzyme Conjugate EK, 12 ml**, ready for use, contains horseradisch-peroxidase conjugate to streptavidin, Use 100 µl for each well in the assay.
7)	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.
8)	SUBST	**Substrate (S), 12 ml**, ready for use, horseradish-peroxidase-(HRP)-substrate,
9)	H_2SO_4	**Stopping Solution (SL), 12 ml,** ready for use, 0.2 M sulphuric acid, Caution acid!
10)		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 research and professional use only.

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

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

Caution: This kit contains material of human and/or animal origin. Therefore all components and patient´s specimens should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.

Human Serum
Following components contain human serum: **Control Sera KS1, KS2**
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, EK, VP, WP, A-E
Contain as preservatives **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 enzyme immunoassay for Progranulin E103 is a so-called Sandwich-Assay. It utilizes specific and high affinity monoclonal antibodies for this protein. The Progranulin in the samples binds to the immobilized first antibody on the microtiter plate. In the following step, the biotinylated antibody binds in turn to Progranulin. After washing, Streptavidin-Peroxidase-Enzyme conjugate will be added, which will bind highly specific to the biotin and will catalyse the enzymatic reaction, which turns the colour of the substrate, quantitatively depending on the Progranulin level of the samples.

SPECIMEN

Serum and plasma samples can be used in this assay. No influence of 3.8 g/l Citrate, 5.4 mmol/l EDTA nor 30 IE/ml Heparin were shown on the measurement of Progranulin by the recovery experiments.

Storage of the samples

Storage at RT	max. 3 days
Storage at +4°C	max. 3 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 95 to 101% of the target value were found.

Sample Preparation

Samples have to be diluted in Dilution Buffer (VP). For most of the determinations (serum or plasma samples, and no extreme values are expected) a serum or plasma dilution **of 1:41 with Dilution Buffer VP** should be suitable. According to expected Progranulin levels the dilution with VP can be higher or lower. The excellent linearity of this test system allows sample dilution of 1:20 to 1:320 (see table 6).

Progranulin 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 **400 µl Dilution Buffer VP** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **10 µl Serum- or Plasma** (dilution1:41). After mixing use 50 µl per determination of this dilution in the assay.

TECHNICAL NOTES
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 opening is not affected, if used appropriately.

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

Incubation at room temperature means: Incubation at 20-25°C
The incubation steps should be performed at mean rotation frequency of a particularly suitable 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 **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 standards 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 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 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
When performing the assay, the Standards **A-E,** Control Sera **KS1& KS2** and the samples should be pipetted as fast as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times, the Enzyme Conjugate **EK** as well as the succeeding **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 **S**
All determinations (Standards, Control Sera and samples) should be assayed in duplicate.
For optimal results, accurate pipetting and adherence to the protocol are recommended.

1) Add **50 µl Antibody Conjugate AK** in **all** wells used.

2) Pipette in positions A1/2 **50 µl Dilution Buffer VP**

3) Pipette in positions B1/2 **50 µl of the Standard A (75 pg/ml),**

   pipette in positions C1/2 **50 µl of the Standard B (250 pg/ml)**,

   pipette in positions D1/2 **50 µl of the Standard C (750 pg/ml)**,

   pipette in positions E1/2 **50 µl of the Standard D (1500 pg/ml)**,

   pipette in positions F1/2 **50 µl of the Standard E (2500 pg/ml)**.

   To control the correct accomplishment of the assay **50 µl** of the 1:41 (or in respective dilution ratio of the samples) in Dilution Buffer VP diluted **Control Sera KS1/KS2** can be pipetted in positions G1/2 and H1/2.

   Pipette **50 µl** each of the diluted samples (e.g. dilute 1:41 with **Dilution Buffer VP**) in the rest of wells, according to your requirements.

4) Cover the wells with sealing tape and incubate the plate for **1 hour** at **room temperature** (shake at ≥350 rpm)

5) After incubation aspirate the contents of the wells and wash the wells **5 times 300 µl Washing Buffer WP** / well.

6) Following the last washing step pipette **100 µl** of the **Enzyme Conjugate EK** in each well.

7) Cover the wells with sealing tape and incubate the plate for **30 Minutes** at **room temperature** (shake 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 **Substrate Solution S** in each well.

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

11) Stop the reaction by adding **100 µl 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 Progranulin:

Standard	**A**	**B**	**C**	**D**	**E**
ng/ml	0.075	0.25	0.75	1.5	2.5
pg/ml	75	250	750	1500	2500

1) Calculate the mean absorbance (MA) value for the blank from the duplicated determination (well A1/A2).
2) Subtract the mean absorbance (MA) 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 **Progranulin 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 Progranulin concentrations of the **undiluted samples** and of control sera are calculated **by multiplication with the respective dilution factor**.

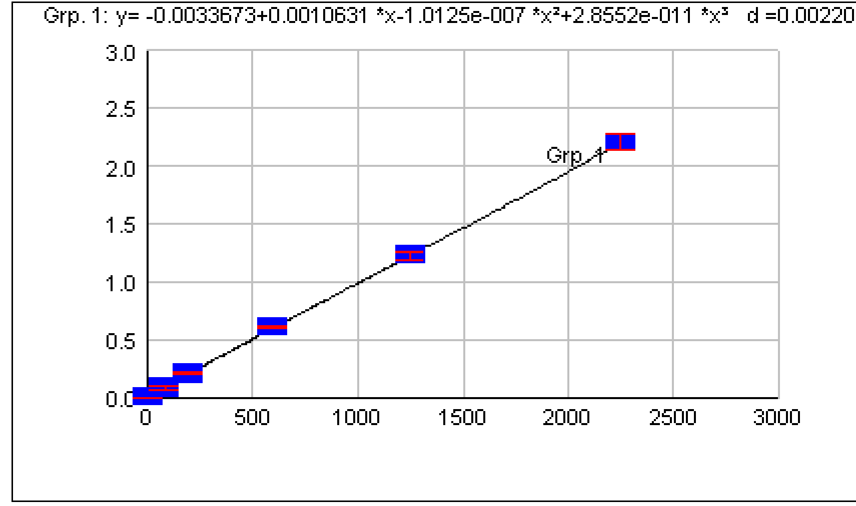

Fig. 1. Exemplary Standard Curve with a polynomial 3 as curve fit.

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 Progranulin concentration of a 1:41 diluted sample:

Measured extinction of your sample 0.56
Measured extinction of the blank 0.03

Your measurement program will calculate the Progranulin concentration of the diluted sample automatically by using the difference of sample and blank (0.03) 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 Progranulin concentration in the sample:

$$0.53 = -0.0033673+0.0010631x-1.0125 \times 10^{-7} x^2+2,8552x \, 10^{-11} \times x^3$$

$$0.5145 = x$$

if the dilution factor (1:41) is taken into account, the Progranulin concentration of the undiluted sample is

$0.5145 \times 41 = 21.10$ ng/ml

PERFORMANCE CHARACTERISTICS

Standards

The standards are prepared from recombinant human Progranulin in concentrations of 75, 250, 750, 1500 and 2500 pg/ml (pico gram/ml, equal to 0.075 -2.5 nano gram/ml).

Sensitivity

The **analytical sensitivity** of the assay yields **0.018 ng/ml** (pg/ml; as 2x SD of zero standard in 19fold determination).

Specificity

Commercially available sera from bovine, cat, chicken, dog, donkey, goat, guinea pig, horse, mouse, pig, rabbit, rat and sheep were diluted 1:5 und 1:41 and used as samples in this assay system and the signal intensity was measured. No cross reactivity was detected.

Recovery

The recovery of recombinant Progranulin in serum and plasma samples varied from 91 to 101%.

Matrix effects

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

Matrix effects						
Dilution [1:x]	**2**	**5**	**10**	**20**	**40**	**100**
Saliva	> max.	> max.	102 %	-	-	-
Urine	106 %	102 %	107 %	-	-	-
Breast milk	> max.	> max.	> max.	> max.	> max.	108 %
Cell culture media	69 %	81 %	91 %	104 %	-	-
Cerebrospinal fluid	73 %	88 %	93 %	-	-	-
Amnion fluid	> max.	> max.	> max.	> max.	102 %	100 %

- = not determined

Interference

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

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

	Triglycerides [100 mg/ml]	**Bilirubin** [200 µg/ml]	**Haemoglobin** [1 mg/ml]
%	104	104	117

Effects of coagulation inhibitors were investigating by adding indicated amounts of inhibitors to VP or PBS enriched with 1250 pg/ml Progranulin. Relative amounts of Progranulin determined in inhibitor containing samples in comparison to inhibitor free samples are shown. None of the tested substances interfered significantly with Progranulin measurement.

Table 3: Effects of coagulation inhibitors.

		Recovery %
[3.8 g/l]	Citrate	95
[5.4 mmol/l]	EDTA	93
[30 IE/ml]	Heparin	98

None of the tested substances interfered significantly with Progranulin measurement.

Reproducibility and Precision

The inter and intra assay coefficients of variability are **below 8.0 and 4.4 %**, respectively.
Exemplary determinations are shown in table 4 and table 5.

Table 4: Inter-Assay-Variation (results of 14 independent determinations)

	Mean (ng/ml)	**Standard deviation (ng/ml)**	**VC (%)**
Sample 1	36.78	2.49	6.76
Sample 2	23.40	1.87	7.99
Sample 3	21.52	1.37	6.36

Table 5: Intra-Assay-Variation

	Number of determinations	**Mean value (ng/ml)**	**Standard deviation (ng/ml)**	**VC (%)**
Sample 1	19	25.61	0.87	3.38
Sample 2	19	49.74	2.17	4.35

Linearity

The Mediagnost Progranulin ELISA E103 is over a very wide range dilution authentic. The linearity of serum dilutions is over a very wide range excellent (see table 6).

Table 6: Linearity of the sample dilution USE103(characteristic result of three different sera)

Dilution	Sample 1 [ng/ml]	Sample 2 [ng/ml]	Sample 3 [ng/ml]
1:20	21.12	14.34	40.56
1:40	23.58	14.08	45.95
1:80	22.17	15.14	46.17
1:160	20.64	16.08	46.89
1:320	19.53	15.59	47.65
AV / 1SD / VC%	**21.41 / 1.54 / 7.20**	**15.05 / 0.84 / 5.57**	**45.44 / 2.81 / 6.18**

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

EXEMPLARY VALUES

Concentrations of Progranulinin human sera of 40 healthy adult donors, at the age of 20 to 65 were determined with the Mediagnost ELISA E103. The concentrations of all samples varied from minimal 21.85 ng/ml to maximal 53.22 ng/ml (see table 7).

Table 7: Expectation values for adults in serum

Gender	Number of samples	Median [ng/ml]	Average value [ng/ml]	Standard Deviation [ng/ml]	Min. – Max.: [ng/ml]
female	20	32.22	31.60	5.62	21.85-40.57
male	20	30.71	33.06	8.11	22.27-53.22
total	40	31.32	32.33	17.35	21.85-53.22

LITERATUR / LITERATURE

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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: 1:41 in Dilution Buffer VP, **mix directly and use within max. 60 min.** Use **50 µl per determination**		
Before assay procedure bring all **reagents** to **room temperature**		

Assay Procedure for Double Determinations:

Pipette	Reagent	Position
50 µl	Antibody Conjugate **AK**	in **all** wells used
50 µl	Dilution Buffer **VP** (blank)	A1 and A2
50 µl	Standard **A** (**75 pg/ml**)	B1 and B2
50 µl	Standard **B** (**250 pg/ml**)	C1 and C2
50 µl	Standard **C** (**750 pg/ml**)	D1 and D2
50 µl	Standard **D** (**1500 pg/ml**)	E1 and E2
50 µl	Standard **E** (**2500 pg/ml**)	F1 and F2
50 µl	Control Serum **KS1**	G1 and G2
50 µl	Control Serum **KS2**	H1 and H2
50 µl	Samples	following wells
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 Wash Buffer WP**	each well
100 µl	Enzyme Conjugate **EK**	each well

Incubation: 30 min at **RT**, **350 rpm**

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

Incubation: 30 min in the dark **RT**

100 µl	Stop Solution **SL**	each well
Measure the absorbance within **30 min** at **450 nm** with **≥ 590 nm** as reference wavelength.		

REF E103 International Test Description

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

Control	1:41 DILU BUF VP
SPE	1:41 DILU BUF VP
°C 20-25 °C	

50 µl	Ab	A1 - End
50 µl	BUF VP	A1/2
50 µl	STD A (75 pg/ml)	B1/2
50 µl	STD B (250 pg/ml)	C1/2
50 µl	STD C (750 pg/ml)	D1/2
50 µl	STD D (1500 pg/ml)	E1/2
50 µl	STD E (2500 pg/ml)	F1/2
50 µl	CONTROL KS 1 1:41 DILU BUF VP	G1/2
50 µl	CONTROL KS 2 1:41 DILU BUF VP	H1/2
50 µl	SPE 1:41 DILU BUF VP	
	TAPE	

À 1 h °C 20-25 ↔ 350 rpm

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

À 0.5 h °C 20-25 ↔ 350 rpm

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

À 30 min °C 20-25

100 µl	H_2SO_4 SL
	MEASURE