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hGH Sensitive ELISA

Enzyme Immunoassay for Quantitative Determination of

human Growth Hormone (hGH)

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

+2 +8°C

96 wells

REF **E022**

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	Lot-Batch Number/ Charge-Chargennummer/ Lot-Code du lot/ Lotto-Numero di lotto/ Lote-Código de lote/ Lote-Código do Lote/Lot-Partijnumme/ Lot-Batchkode/ Partisatskod/ Numer serii/ Tétel-sarzs szám/ Číslo šarže/ Číslo šarže/ Партиден номер/Partii number/ Παρτίδα-αριθμός παρτίδας/ Lot-număr lot/ Številka serije/ Erä
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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/ 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 sluwnečnímu 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
MTP	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
Rec in	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
DET	Antibody Conjugate/ Antikörperkonjugat/ Anticorps conjuguée/ Coniugato di anticorpo/ Conjugado de anticuerpos/ Conjugado anticorpo/ Antilichaamconjugaat/ Antistoffer-konjugat/ Antikroppskonjugat/ Koniugat antyciał/ Antitest páros/ Protilátkový konjugát/ Protilátkový konjugát/ Антитяло конюгат/ Antikehad konjugaat/ Σύμπλοκο αντισώματος/ Compuşi din anticorpi/ Antitelesa konjugat/ Vasta-aine konjugaatti
EC	Enzyme Conjugate/ Enzymkonjugat/ Conjugué enzymatique/ Coniugato di enzima/ Conjugado de enzimas/ Conjugado Enzima/ Enzymconjugaat/ Enzym-konjugat/ Enzymkonjugat/ Koniugat enzymów/ Enzim páros/ Enzymatický konjugát/ Enzymatický konjugát/ ензим конюгат/ Ensüümi konjugaat/ Σύμπλοκο –ενζύμου/ Compuşi din enzime/ Encima konjugat/ Entsymi konjugaatti
DIL	Dilution Buffer/ Verdünnungspuffer/ Tampon de dilution/ Tampone di diluizione/ Tampón de dilución/ / Tampão de diluição / Verdunningsbuffer/ / Fortyndingsbuffer/ Utspädningsbuffert / Bufor rozcieńczający/ / Hígító puffer/ Riediaci pufor/ Ředicí pufr / Буфер за разреждане/ Lahjenduspuhver/ Ρυθμιστικό διάλυμα αραίωσης / Tampon de diluare/ Pufer za redčenje/ Laimennuspuskuri

X:X	Dilute / Verdünnen / Diluer / Diluire / Diluir / Diluir / Verdunnen / Fortyndes / Späd / Rozcieńczanie / Hígítás / Riediť /Ředit / Разреждане / Lahjendada / Αραιώστε / Diluaţi / Razredčiti / Laimennetaan
CAL A-E	Calibrator X/ Kalibrator X/ calibrateur X/ calibratore X/ calibrador X/ calibrador X/ kalibrator X/ kalibrator X/ kalibrator X/ kalibrator X/ kalibrátor X/ kalibrátor X/ калибратор X/ kalibraator X/ Βαθμονομητής X/ calibrator X/ kalibrator X/ kalibraattori X
CTR	Control/ Kontrolle/ Contôle/ controllo/ control/ Controle/ controle/ Kontrol/ Kontroll/ kontrolne/ Ellenőrző/ Kontrolné/ Kontrolní/ Контролен/ Kontroll/ ελέγχου/ control/ Kontrolni/ Kontrolli
WB	Washing Buffer Concentrate/ Waschpufferkonzentrat/ Tampon de lavage conc./ Tampone di lavaggio concentrato/ Tampón de lavado concentrado/ Tampão de Lavagem Concentrado/ wasbuffer, geconcentreerd/ Vaskebufferkoncentrat/ Vaskebufferkoncentrat/ tvättbuffertkoncentrat/ Bufor płukania koncentrat/ Mosópuffer koncentrátum/ Koncentrát vymývacieho pufra/ Концентрат на промивен буфер/ Pesupuhvri kontsentraat/ Συμπύκνωμα ρυθμιστικού διαλύματος έκπλυσης/ Concentrat pentru tamponul de spălare/ Koncentrat izpiralnega pufra/ Pesuliuositiiviste
WB 1:20	Washing Buffer/ Waschpuffer/ Tampon de lavage/ Tampone di lavaggio/ Tampón de lavado/Tampão de Lavagem/ wasbuffer/ Vaskebuffer/ tvättbuffert/ Bufor płukania/ Mosópuffer/ Vymývací pufer/ Vymývací pufr/ Промивен буфер/ Pesupuhver/ Ρυθμιστικό διάλυμα έκπλυσης/ Tampon pentru spălare /Izpiralni pufer/ Pesuliuos
S	Substrate/ Substrat/ Substrat/ Substrato/ Substrato/ Substrato/ substraat/ Substrat/ Substrat/ Substrat/ Szubsztrátum/ Substrát/ Substrát/ Субстрат Substraat/ Υπόστρωμα/ Substrat/ Substrat/ Substraattiliuos
STP	Stop Solution/ Stopplösung/ Stop Solution/ Soluzione di stop/ Stop Solución/ Solução Stop/ stopoplossing/ Stopopløsning/ Stopplösning/ Stop roztwór/ Megállító oldat/ Roztok na ukončenie/ Roztok pro ukončení/ Стопиращ разтвор/ Stopp-lahus/ Διάλυμα διακοπής/ Soluţie de oprire/ Stop raztopina/ Pysäytysliuos
TAPE	Cover Plate with sealing tape/ Platte abkleben/ Recouvrir la microplaque avec bande adhésive/ Coprire la piastra con nastro adesivo/ Cubrir la placa con una cinta adhesiva/ Cobrir a Placa com fita adesiva/ plaatje met tape afdekken/ Afdækningsplade med tape/ maskera platta/ Odkleić płytkę/ Tányér leragasztása/ Oblepiť podložku lepiacou páskou/ Olepit podložku lepicí páskou/ Плака с лента за запечатване/ Katta plaat isoleerkleeplindiga/ Κολλήστε το πλακίδιο με κολλητική ταινία/ Aoperiţi placa cu o bandă adezivă/ Prelepiti ploščo/ Peitä mikrotitrauslevy oheisella teipillä
MEASURE	Measure plate within 30 min at 450 nm (Referencefilter ≥590 nm)/ Ausmessung innerhalb von 30 min bei 450 nm (Referenzfilter ≥ 590 nm)/ Mesure lábsorbance en léspace de 30 min à 450 nm avec ≥590 nm longueur dónde pour référence/ Misurazione entro 30 min. a 450 nm (filtro di riferimento ≥ 590 nm)/ Medición de la placa dentro de los siguientes 30 min a 450 nm (filtro de referencia ≥ 590nm)/ Medir a placa dentro de 30 min a 450 nm (Filtro de referência ≥ 590 nm)/ Binnen 30 minuten bij 450 nm meten (referentiefilter ≥ 590 nm)./ Mål plade i løbet af 30 min ved nm (referencefilter ≥590 nm)/ Mät inom 30 min vid 450 nm (referensfilter ≥ 590 nm)./ Pomiar w ciągu 30 min przy 450 nm (filtr odniesienia ≥ 590 nm)/ Ki mérés 30 percen belül 450 nm-nél (referenciaszűrő ≥ 590 nm)/ Merať 30 minút pri 450 nm (Referenčných filtrov ≥590 nm)/ Měřit 30 minut při 450 nm (Referenční filtr ≥ 590 nm)/ Отчитане в рамките на 30 min при 450 nm (референтен филтър ≥ 590 nm)/ Mõõtmine 30 min jooksul 450 nm korral (võrdlusfilter ≥ 590 nm)/ Μέτρηση εντός 30 min στα 450 nm (φίλτρο αναφοράς ≥ 590 nm)/ Măsurare în decurs de 30 min la 450 nm (filtru de referinţă ≥ 590 nm)/ Izmerite ploščico v 30 min pri 450 nm (referenčni filter ≥590nm)/ Mittaa 30 minuutin aikana 450 nm:ssä (referenssi suodatin ≥ 590 nm)
Literature	Literature/ Literatur/ Bibliographie/ Letterario/ Bibliografía/ Literatura documentação/ literatuur/ Litteratur/ litteratur/ Literatura/ Irodalom/ Literatúra/ Literatura/ Литература// Kirjandus/ Βιβλιογραφία/ Bibliografie/ literatura/ Lähdeluettelo
International Test description	International test description/ internationale Testanleitung/ description internationale de test/ Istruzioni per il test internazionali/ Descripción de ensayo internacional/ Descrição internacional do teste/ internationale testbeschrijving/ internationell testbeskrivning/ Opis testu międzynarodowego/ nemzetközi teszt-útmutató/ Medzinárodný návod k testu/ Mezinárodní návod k testu/ rahvusvaheline katse kirjeldus/ Διεθνείς οδηγίες για εργαστηριακές δοκιμές/ instrucţiuni internaţionale pentru testare/ mednarodna navodila za preizkus/ Kansainvälinen käyttöohje
End	in all required wells/ in allen benötigten Vertiefungen/ dans tous les godets requis/ in tutti i pozzetti richiesti/ en todos los pozos requeridos/ em todos os tubos necessários/ in alle nodige putjes/ i alle nødvendige brønde/ i alla nödvändiga brunnar/ we wszystkich potrzebnych wgłębieniach/ minden szükséges forrásban/ vo všetkých potrebných miestach/ ve všech potřebných místech/ във всички необходими ямки/ kõigis vajalikes süvendites/ σε όλες τις απαραίτητες κοιλότητες/ în toate cavităţile necesare/ v vseh zahtevanih vdolbinah/ kaikkiin tarvittaviin mikrotitrauslevyn syvennyyksiin

TABLE OF CONTENTS

Instructions for use

hGH sensitive ELISA	96 Determinations
Principle of the test	Sandwich ELISA
Duration (incubation period)	3.25 h
Antibody Conjugate	ready for use
Enzyme Conjugate	ready for use
Dilution Buffer and Substrate	ready for use
Washing Buffer	20x concentrate
Reference material	2. International Standard WHO/ NIBSC 98/574, recombinant hGH
Calibrators	5 single calibrators: 0.05 - 1 ng/mL, lyophilized, recombinant hGH
Assay Range	0.0115 - 26 ng/mL
Control	1 Control, freeze-dried
Sample	human serum / plasma
Required sample volume	10 µL
Sample dilution	1:26
Analytical sensitivity	ø 0.0115 ng/mL
Intra- / Inter-Assay Variance	ø < 10%

1 INTENDED USE

This enzyme immunoassay kit is for research use and quantifies hGH in human Serum, Heparin or EDTA plasma.

2 INTRODUCTION

The endocrine system of human Growth Hormone (hGH), also named Somatropin, is characterized by an extreme complexity. hGH is the product of the GH-1 gene located on chromosome 17 and expressed in pituitary cells. 80% of the hGH is a non-glycolsylated 22 kDa protein consisting of 191 amino acids. About 20% is a variant form of 20 kDa resulting from alternative splicing. Additionally, several smaller variants can be found in circulation as well as translational modificated proteins and different degrees of protein aggregation. Bioactivity of Growth Hormone is regulated by a specific binding protein (GHBP) formed by the extra cellular part of the cellular transmembran GH-receptor. These modifications allow a tight control of the half-life period hGH and of its bioactivity.

Not only synthesis and posttranslational modification but also secretion of hGH is tightly regulated. Spontaneous pulsatile secretion takes place with a single pulse every three hours and a maximal secretion during night’s sleep. Several different stimuli as physiologic stress or hypoglycaemia result in additional hGH secretion, induced by the hypothalamic hormones Somatostatin and GH-Releasing Hormone (GHRH). The amount of secreted GH is influenced by multitude of factors (see References 2-5).

3 ASSAY PRINCIPLE

The Mediagnost hGH SENSITIVE ELISA E022 is a so-called sandwich-assay. It utilizes a specific, high affinity polyclonal rabbit antiserum coated on the wells of a microtiter plate. The hGH in the samples binds quantitatively to the immobilized antiserum. In the following step, the biotinylated antibody in turn binds hGH. After washing, a streptavidin-peroxidase-enzyme conjugate will be added, which will bind highly specific to the biotin of the antibody and will catalyze the substrate to change the color quantitatively depending on the hGH level of the sample.

4 WARNINGS AND PRECAUTIONS

For Research Use only. Not for use in diagnostic procedures. For Professional use only.
The Mediagnost kit is suitable only for in vitro **Research 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 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 CTR**
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 specimens should be treated as potentially infectious.

Reagents A-E, DET, EC, DIL, WB
Contain as preservative a mixture of **5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one** (<0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

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

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.

Stop Solution STP
The Stop Solution contains 0.2 M acid sulphur acid (H_2SO_4)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+	IF SWALLOWED: rinse mouth.
P331	Do NOT induce vomiting.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

4.1 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.

5 SAMPLES

5.1 Sample type

Serum and Plasma
Serum and Heparin/EDTA Plasma yield comparable values. The hGH levels are reduced in citrate plasma samples, because of the relatively high amount of anticoagulant.
In addition to serum and plasma samples hGH can be determined in other human body fluids like urine and saliva, in cell culture supernatants of various human cell lines for research purposes. Additionally this hGH Sensitive ELISA E022 can be used for measurement of hGH in filter paper samples, which has been validated externally (9).

5.2 Specimen collection

Human GH is secreted pulsatile during the day/night.

Use standard venipuncture for the blood sampling. Haemolytic reactions are to be avoided.

5.3 Required sample volume: 10 µL

5.4 Sample stability

In firmly closable sample vials

- Storage at 20-25°C: 3 days
- Storage at 20°C: min. 2 years
- Freeze-thaw cycles max. 5

The storage of samples over a period of 2 years at -20°C, showed no influence on the reading. Freezing and thawing of samples should be minimized. 5 Freezing-Thawing showed no effect on samples.

5.5 Interference

Triglyceride and bilirubin in the sample do not interfere to a concentration of 100 mg/mL and 200 µg/mL. However, the use of hemolytic, lipemic or icteric samples should be validated by the user.

5.6 Sample dilution

- Serum/ Plasma Dilution: **1:26** with Dilution Buffer **DIL**

  Pipette **250 µL** Dilution Buffer **DIL** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series); add **10 µL sample** (dilution 1:26). After mixing use 2 x 100 µL of this dilution in the assay.

- Sample stability after dilution of the sample: at least 1 hour at **20-25°C**.

- In the other samples than serum and plasma, the hGH levels can vary considerably, the optimal dilution must be found out by the customer.

6 MATERIALS

6.1 Materials provided

The reagents listed below are sufficient for 96 wells including the calibration curve

MTP	**Microtiter plate,** ready for use, coated with rabbit-anti-hGH-antibody. Wells are separately breakable.	**(8x12) wells**
CAL A-E	**Calibrators**, lyophilised, (recombinant human hGH), concentrations are given on vial labels and on quality certificate in	**5 x 750 µL**
CTR	**Control,** lyophilised, (human serum), concentration is given on quality certificate in ng/mL.	**1 x 500 µL**
DET	**Antibody Conjugate,** ready for use, contains rabbit biotinylated anti-hGH antibody**.**	**1 x 12 mL**
EC	**Enzyme Conjugate,** ready for use, contains HRP (Horseradish-Peroxidase)-labelled Streptavidin.	**1 x 12 mL**
DIL	**Dilution Buffer,** ready for use	**1 x 120 mL**
WB	**Washing Buffer,** 20-fold concentrated solution	**1 x 50 mL**
S	**Substrate,** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised Tetramethylbencidine.	**1 x 12 mL**
STP	**Stop Solution,** ready for use, 0.2 M sulphuric acid.	**1 x 12 mL**
-	**Sealing Tape,** for covering the **microtiter plate.**	**2 x**
	**Instructions for use**	**1 x**
-	**Quality Certificate**	**1 x**

6.2 Materials required, but not provided

- Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer **WB (A. dest.),** 950 mL.
- Precision pipettes and multichannel pipettes with disposable plastic tips
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Vortex-mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)
- Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm

7 TECHNICAL NOTES

Storage Conditions

Store the kit at **2-8°C**.after receipt until its expiry date. The lyophilized reagents should be stored at –20 °C after reconstitution. Avoid repeated thawing and freezing.

Storage Life

The shelf life of the components **after initial opening** is warranted for **4 weeks**, store the unused strips and microtiter wells **airtight** together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The **reconstituted components** calibrators **A-E** and Control **CTR** must be stored at **-20°C**: (max. 4 weeks). For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Up to 3 of the freeze-thaw cycles did not influence the assay. The 1:20 diluted Washing Buffer **WB** is 4 weeks stable at **2-8°C**.

Preparation of reagents

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. Reagents with different lot numbers cannot be mixed. Temperature WILL affect the absorbance readings of the assay. However, values for the samples will not be affected.

Reconstitution

The Calibrators **A – E** and Control **CTR** are reconstituted with the Dilution Buffer **DIL**. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

Dilution

After reconstitution dilute the Control **CTR** with the Dilution Buffer **DIL** in the same ratio (1:26) as the sample.
The required volume of Washing Buffer **WB** is prepared by 1:20 dilution of the provided 20fold concentrate with Aqua dest.

Assay Procedure

When performing the assay, Blank, Calibrators **A-E**, Control **CTR** and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Antibody Conjugate **DET** and the Enzyme Conjugate **EC** as well as the succeeding Substrate **S** should be added to the plate in the same order and in the same time interval as the samples. Stop Solution **STP** should be added to the plate in the same order as Substrate **S**.
All determinations (Blank, Calibrators **A-E**, Control **CTR** and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

Incubation

Incubation at room temperature means: Incubation at 20-25°C. The Substrate **S**, stabilised Tetramethylbencidine, is photosensitive–store and incubation in the dark.

Shaking

The incubation steps (except the 3. incubation step) should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm., the incubation after addition of Enzyme Conjugate EC is without shaking. Due to certain technical differences deviations may occur, in case the rotation frequency must be 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.

Washing

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 **WB** 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 dynamical 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.

8 ASSAY PROCEDURE

All determinations **(Blank**, **Calibrators A-E**, **Control CTR and samples)** should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

When performing the assay **Blank**, **Calibrators A-E**, **Control CTR** and the **samples** should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, **Antibody Conjugate DET** and the **Enzyme Conjugate EC** as well as the succeeding **Substrate S** should be added to the plate in the same order and in the same time interval as the samples. **Stop Solution STP** should be added to the plate in the same order as **Substrate S**.

1) Add **100 µL Dilution Buffer DIL** in wells **A1/A2** (blank).
2) Pipette in positions **B1/2 100 µL Calibrator A (0.05 ng/mL)**
   Pipette in positions **C1/2 100 µL Calibrator B (0.15 ng/mL)**
   Pipette in positions **D1/2 100 µL Calibrator C (0.3 ng/mL)**
   Pipette in positions **E1/2 100 µL Calibrator D (0.6 ng/mL)**
   Pipette in positions **F1/2 100 µL Calibrator E (1 ng/mL)**
   For control purpose pipette 100 µL of the 1:26 (or respective dilution as the sample) diluted Control in positions G1/2.
   Pipette **100 µL of each of the diluted samples** (e.g., diluted 1:26 or other) into the rest of the wells.
3) Cover the wells with sealing tape and incubate the plate for **2 hours at room temperature 20 - 25°C** (shake at 350 rpm).
4) After incubation aspirate the contents of the wells and wash the wells 5 times with **300 µL Washing Buffer WB**/ well. Aspirate wells after each washing. Following the last washing step, bang the plate inverted onto a paper towel to remove residual liquid.
5) Pipette **100 µL of the Antibody Conjugate DET** into each well.
6) Cover the wells with sealing tape and incubate the plate for **0.5 hour** at room temperature (shake at 350 rpm).
7) Subsequently –**without a washing step!** - pipette **100 µL** of the **Enzyme-Conjugate EC** in each well and incubate additional **30 minutes without shaking.**
8) After incubation wash the wells **5 times** with **Washing Buffer WB** as described in step 4.
9) Pipette **100 µL of the Substrate S** in each well.
10) Incubate the plate for **15 minutes in the dark at room temperature 20 - 25°C**
11) Stop the reaction by adding **100 µL of Stop Solution STP**.
12) Measure the colour reaction within 30 minutes at **450 nm**
    (**reference filter ≥590 nm**).

9 EVALUATION OF RESULTS

9.1 Establishing of the calibration curve

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.25, and the absorbance of Calibrator E should be above 1.00.
Samples, which yield higher absorbance values than Calibrator E, should be re-tested with a higher dilution.
The 2nd International Standard for hGH, NIBSC Code 98/574 (6), was used as calibrator material. This was defined in an international study in the year 2001 with 3 International units per mg Protein (3 IU/mg). The exclusive application of this calibrator material is recommended in line with the current standardisation efforts for hGH Immunoassays. (7,8)

Calibrator	A	B	C	D	E
ng/mL	0.05	0.15	0.30	0.60	1.0
pg/mL	50	150	300	600	1000
µIU/mL	0.15	0.45	0.9	1.8	3.0

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 samples, control and calibrators.
3) Plot the calibrator concentrations on the x-axis versus the mean value of the absorbance of the calibrators on the y-axis.
4) Recommendation: Calculation of the calibration curve should be done by using a computer program, because the curve is in general (without respective transformation) not ideally described by linear regression. **A higher-grade polynomial, or four parametric logistic (4-PL) curve fit** or **non-linear regression** are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).
5) The hGH concentration in ng/mL (or pg/mL, or µIU/mL, according the chosen unit for the calibrators) of the samples and control can be calculated by **multiplication** with the respective **dilution factor.**

9.2 Example of a typical calibration curve

The following data is for demonstration only and cannot be used in place of data generation at the time of assay.

Calibrator	Blank	A	B	C	D	E
ng/mL	0.0	0.05	0.15	0.3	0.6	1.0
OD $_{(450\text{-}620\ nm)}$	0.08	0.2955	0.5885	1.1045	1.9935	2.761

The exemplary shown calibration curve in Figure 1 **cannot** be used for calculation of your test results. You have to establish a calibration curve for each test you conduct!

9.3 Exemplary calculation of GH concentrations

Sample dilution: 1:26

Measured extinction of your sample 0.25
Measured extinction of the blank 0.08

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

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

$$0.17 = -1.2868x^3 + 0.8619x^2 + 3.0523x + 0.0532$$
$$0.035 = x$$

If the dilution factor (**1:26**) is taken into account the hGH concentration of the undiluted sample is

$$0.035 \text{ ng/mL} \times 26 = 0.91 \text{ ng/mL}$$

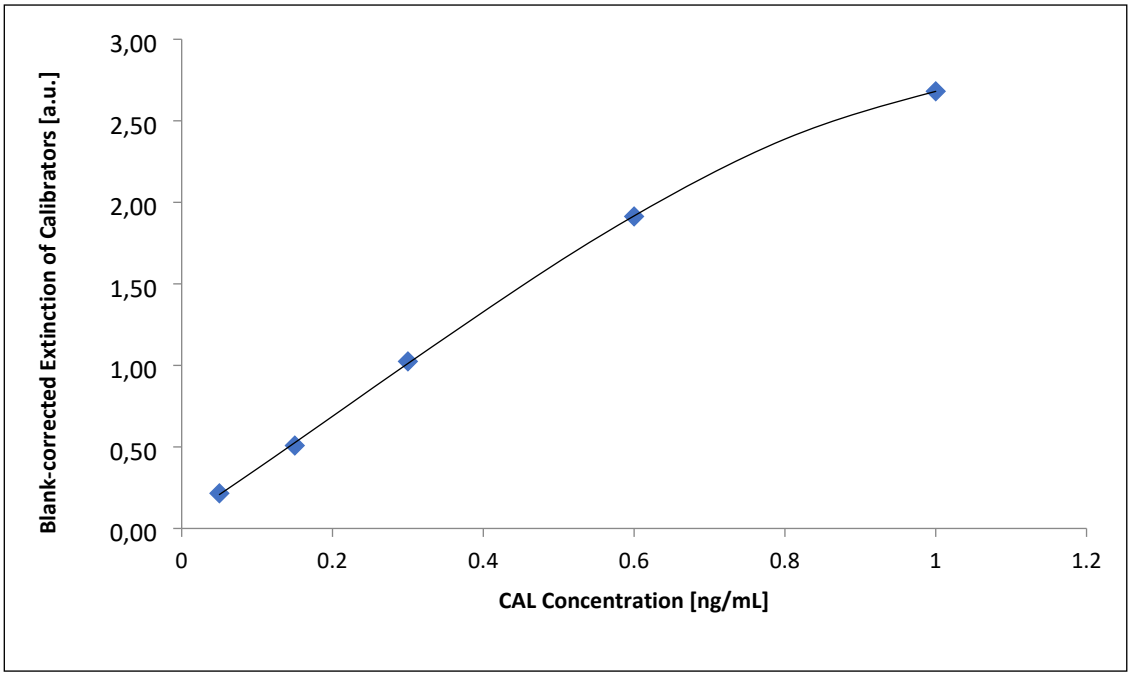

Figure 1: Examplary calibration curve

9.4 Interpretation of results

As the used test system is calibrated to WHO standard 98/574 a secretion peak of less than **8 ng/mL** is a non-binding benchmark.

9.5 Limitation of procedure

The Mediagnost sensitive human Growth-Hormone ELISA, E022 is based on polyclonal rabbit antibodies. Generally, this technique is sensible to heterophilic antibodies in the sample. The influence of heterophilic antibodies is reduced by assay design, but cannot be excluded completely.
Interference of several physiological substances has been tested for the indicated concentrations. Higher concentrations or other substances may interfere with the measurement.

9.6 Examplary values

We investigated hGH serum concentration of 104 healthy blood donors in the age of 18-69 years without any stimulation and undefined sampling time.

Table 1: Exemplary values hGH serum concentration of 104 healthy blood donors.

	female	**male**
number	54	50
median [ng/mL]	0.81	0.28
minimal concentration [ng/mL]	0.19	0.15
maximal concentration [ng/mL]	10.45	4.34

10 PERFORMANCE CHARACTERISTICS

10.1 Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the E022 is ø 0.0115 µg/L.

10.2 Specificity

Cross reactivity with recombinant human Prolactin has been tested and no significant signal was measured in an enriched serum sample containing 200 µg/L Prolactin.

Further, Pegvisomant (trade name Somavert), a growth hormone analogue has been tested for cross-reactivity in assay buffer in different concentrations. Here no significant influence of Pegvisomant was detected (Table 2). However, a study (9) showed that, after the addition of Pegvisomant (100 mg/L) to serum samples with enriched with hGH (2.6 and 10.1 µg/L), the measured concentrations of hGH were 154% and 108% of the expected values.

Table 2: To determine the **specificity** Pegvisomant (trade name Somavert) was diluted in the Dilution Buffer (DIL) at the indicated concentrations and used as a sample in E022. The cross-reactivity of Pegvisomant is presented.

Pegvisomant [mg/L]	Concentration measured in E022 [mg/L]	% Cross-reactivity
100	0.0114	0.0114
10	0.00845	0.0845
1	0.00436	0.436
0.1	0.00103	1.03
0.01	0.00017	1.7
0.001	0.00006	6
0.0001	0.00004	40

10.3 Precision

Intra-Assay-Variation

One sample has been measured 14 times in the same assay. The results are shown in Table 3. The measured coefficient of variation (CV) is 5.46%. Intra assay variance has also been evaluated externally (9), two serum samples with 0.45 and 5.94 µg/L hGH were measured 10 times within the same assay. The resulting coefficients of variation were 3.65% and 2.16%

Table 3: Intra-Assay Variation

	Number of determinations	Mean value (µg/L)	Standard deviation (µg/L)	VC (%)
Sample 1	16	2.41	0.19	7.99
Sample 2	16	5.84	0.27	4.70
Sample 3	16	14.98	0.55	3.70

Inter Assay Variance

Serum samples were measured in independent assays. On average the coefficient of variation was 4.34%. Results are shown in detail in table 4. Here also externally acquired data are available: The mean coefficient of variation for inter-assay variance at 2.39; 5.37 and 14.33 µg/L hGH was 5.98%; 3.93% and 3.12%, respectively (9).

Table 4: Inter-Assay Variation

	Number of single determinations	Mean value (µg/L)	Standard deviation (µg/L)	VC (%)
Sample 1	14	5.37	0.21	3.93
Sample 2	10	2.39	0.14	5.98
Sample 3	11	14.33	0.45	3.12

10.4 Linearity

Linearity of the E022 was tested by dilution of 2 different serum samples. The samples were diluted in the range of 1:10 to 1:76800. Linearity of sample dilution was shown by linear regression in the dilution range of 1:10 - 1:9600 (Figure 2).

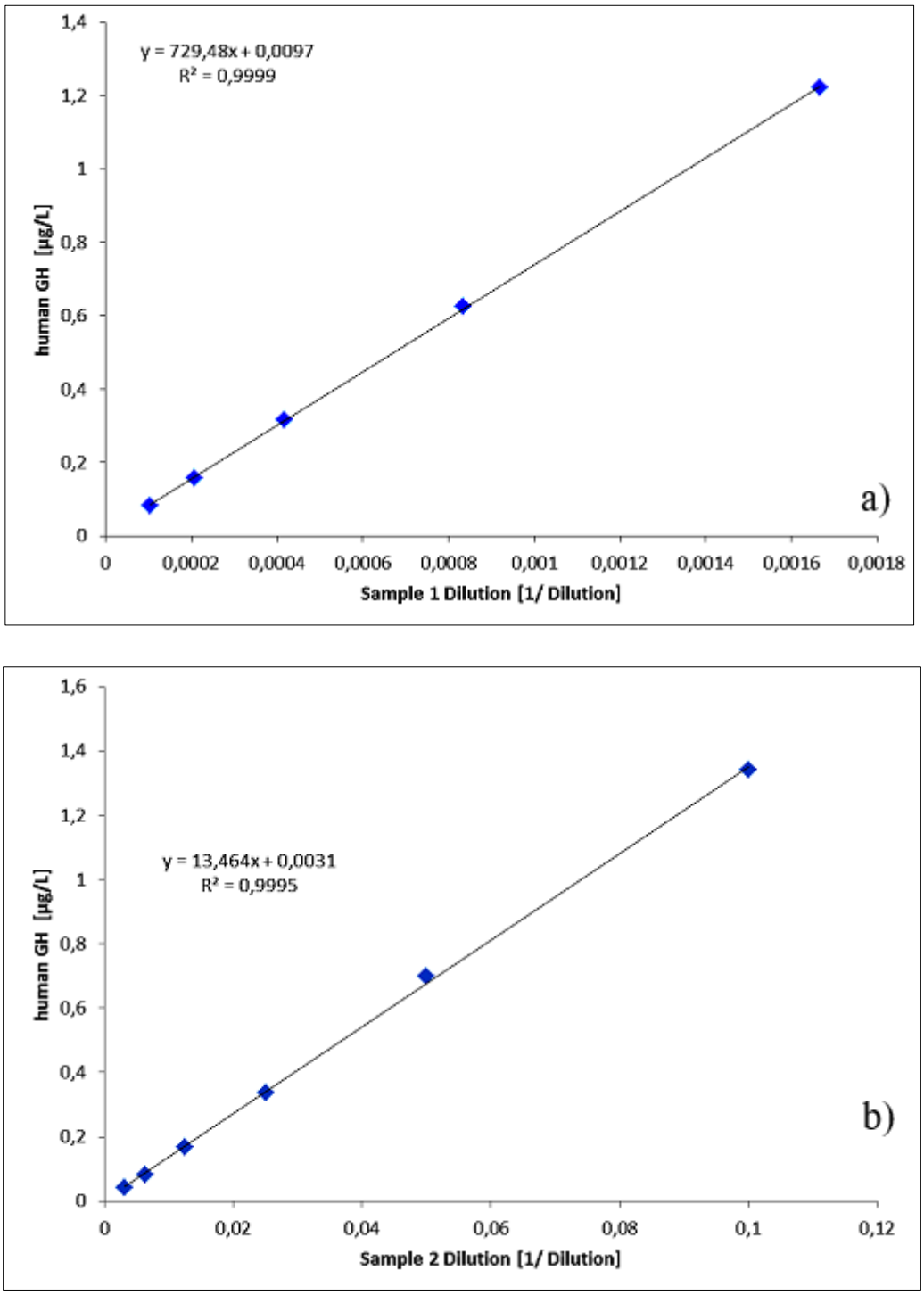

Figure 2: Linearity sample dilution. Two samples with different hGH concentrations were diluted a) 1:600 - 1:9600, b) 1:10 - 1:320. The hGH concentrations were recalculated.

10.5 Recovery and Accuracy

Recombinant human Growth Hormone (NIBSC 98/574) was added in different amounts to human serum. The hGH content of the so enriched samples was measured and recovery in comparison to enriched Dilution Buffer (**DIL**) calculated. Results are shown in table 5.

Table 5 Recovery of recombinant human GH in Serum.

NIBSC Rec. hGH	**DIL**		**Serum 1 PAA539**		**Serum 2 PAA 574**	
ng/mL	ng/mL	%	ng/mL	%	ng/mL	%
20	19.34	96.7	17.51	83.2	17.53	81.9
10	9.81	98.1	9.28	84.0	9.18	80.4
5	5.34	106.7	4.99	82.67	5.9	92.0
0	0	-	1.04	-	1.41	-

10.6 Interference

Interference of bilirubin and triglycerides has been tested in [9]. Here neither bilirubin (up to 200 mg/L) nor triglycerides (up to 100 g/L) showed a significant interference with hGH measurement Table 6. The authors also tested the influence of growth hormone binding protein up to 10 µg/L on hGH measurement and haven´t seen a significant effect (mean recovery 98%). Mediagnost data are shown in Table 7.

Table 6 Interference of GHBP on GH measurement

	hGH [µg/L]		
	2	**8**	**20**
GHBP [µg/L]	1	1	1
hGH Recovery [%]	95	95	99
GHBP [µg/L]	5	5	5
hGH Recovery [%]	87	95	97
GHBP [µg/L]	10	10	10
hGH Recovery [%]	87	93	95

Table 7 Interference of Bilirubin and Triglycerides on GH measurement

Bilirubin [mg/L]	**hGH Recovery [%]**	**Triglycerides [g/L]**	**hGH Recovery [%]**
25	111	12.5	89
50	116	25	109
100	112	50	85
200	108	100	110

10.7 Species Cross-Reactivity

Several commercially available animal sera have been tested as samples in different dilutions (1:5 or 1:26) in this assay.

No signal was detected in serum of the following species: donkey, dog, goat, guinea pig, horse, rat, mouse, rabbit, sheep, cat, chicken and horse.

Whether this obvious non-reactivity is species specific should be assessed individually by each customer. We remind each customer that hGH secretion is pulsatile and thus commercially available animal serum samples may not be taken at the ideal daytime. An external laboratory was able to find good measurable signals in bovine serum.

11 COMPARISON STUDIES

Langkamp et al Growth Horm IGF Res 18 (2008) 526-532 compared an in-house assay used for hGH measurements for years with the Mediagnost E022 (9). Results show a very good comparability and thus cut-off values established by the in house-assay can also be used with the Mediagnost E022 (see Figure 3).

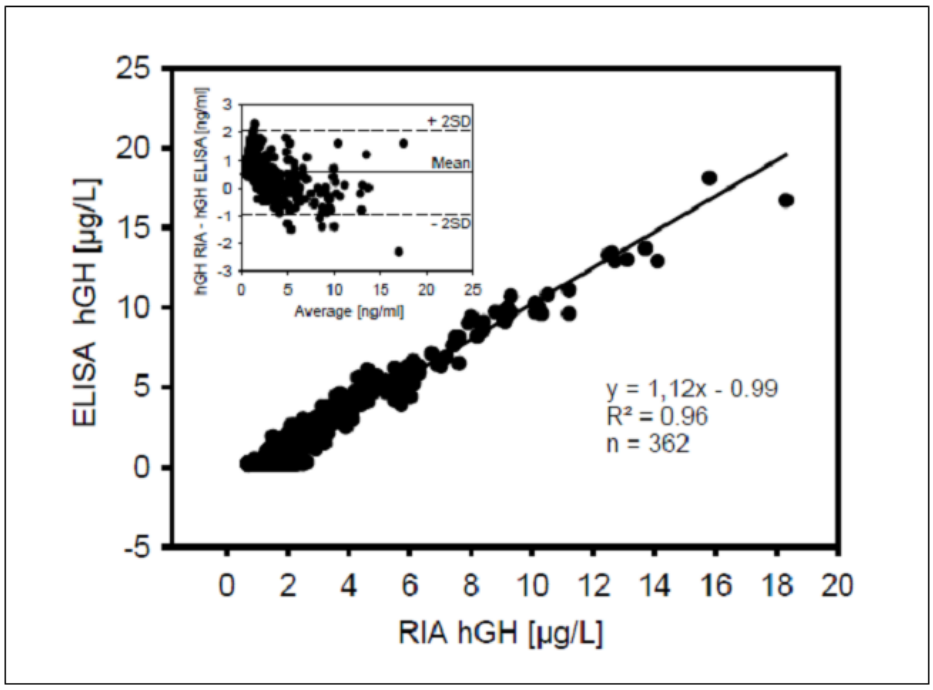

Figure 3: Assay Comparison.

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13 Internationale Assay Description

International Test description

CAL A-E	**Rec in 750 µL DIL**	**-**
CTR	**Rec in 500 µL DIL**	**1:26 DIL**
WB 20x	**-**	**1:20 A. dest.** ➔ **WB 1:20**
SPE		**1:26 DIL**
°C 20-25°C		

100 µL	**DIL**	A1/A2
100 µL	**CAL A (0.05 ng/mL)**	B1/B2
100 µL	**CAL B (0.15 ng/mL)**	C1/C2
100 µL	**CAL C (0.30 ng/mL)**	D1/D2
100 µL	**CAL D (0.60 ng/mL)**	E1/E2
100 µL	**CAL E (1.00 ng/mL)**	F1/F2
100 µL	**CTR 1:26 DIL**	G1/G2
100 µL	**SPE 1:26 DIL**	
	TAPE	
	2 h °C 20-25°C ↔ 350 rpm	
5x 300 µL	**5x WB 1:20**	
100 µL	**DET**	
	TAPE	
	0.5 h °C 20-25°C ↔ 350 rpm	
100 µL	**EC**	
	TAPE	
	0.5 h °C 20-25°C	
5x 300 µL	**5x WB 1:20**	
100 µL	**S**	
	0.25 h °C 20-25°C	
	STP	
	MEASURE	

14 ASSAY PROCEDURE

Preparation of reagents		**Reconstitution:**	**Dilution**
CAL A-E	**Calibrators**	in **750 µL** Dilution Buffer **DIL**	-
CTR	**Control**	in **500 µL** Dilution Buffer **DIL**	**1:26** with **DIL**
WB	**Washing Buffer concentrate.**	-	**1:20** with **Aqua dest.** ➔ **WB 1:20**
Sample dilution: with Dilution Buffer **DIL 1:26**			
Before assay procedure bring all reagents to room temperature **20-25°C**.			

Assay Procedure in Double Determination:

Pipette	**Reagents**	**Position**
100 µL	Dilution Buffer **DIL** (**Blank**)	A1/A2
100 µL	Calibrator **A** (**0.05 ng/mL**)	B1/B2
100 µL	Calibrator **B** (**0.15 ng/mL**)	C1/C2
100 µL	Calibrator **C** (**0.30 ng/mL**)	D1/D2
100 µL	Calibrator **D** (**0.6 ng/mL**)	E1/E2
100 µL	Calibrator **E** (**1.0 ng/mL**)	F1/F2
100 µL	Control **CTR** (1:26 diluted)	G1/G2
100 µL	Sample **SPE** (1:26 diluted)	in the rest of the wells according the requirements
Cover the wells with the sealing tape.		
Sample Incubation: **2 h at 20-25°C**, **350 rpm**		
5 x 300 µL	Aspirate the contents of the wells and **wash** 5 x with 300 µL each Washing Buffer **WB 1:20** **/ well**	In each well
100 µL	Antibody Conjugate **DET**	In each well
Cover the wells with the sealing tape.		
Incubation: 30 Minutes at 20-25°C, 350 rpm		
100 µL	Enzyme Conjugate **EC, without washing the wells (!)** – add to the previously pipetted Antibody Conjugate **DET**-solution **thereto** mix shortly through cautious tapping on the side of the **MTP**. **Attention:** high filled volume of the wells!	In each well
Cover the wells with the sealing tape.		
Incubation: 30 Minutes at 20-25°C, without shaking		
5 x 300 µL	Aspirate the contents of the wells and **wash** 5 x with 300 µL each Washing Buffer **WB 1:20** **/ well**	In each well
100 µL	Substrate **S**	In each well
Incubation: 15 Minutes in the Dark at 20-25°C		
100 µL	Stop Solution **STP**	In each well
Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength.		