hGH ELISA

Enzyme Immunoassay for Quantitative Determination of

human Growth Hormone (hGH)

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

+2°C +8°C

96 wells

REF **E02**

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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
	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
°C	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
	Mix tubes with a Vortex mixer/ Mix Röhrchen mit Vortex Mixer/ Mélanger à l'aide d'un vortex/ Miscelare la provetta con agitatore Vortex/ Tubos de mezcla con mezclador de vórtex/ Misturar os tubos com um agitador Vortex/ buisjes mengen met een Vortex/ Blanderør med Vortex-mixer/ Blanda rören med en vortexblandare/ Miksowanie rurek w mikserze Vortex/ Csövecskék keverése örvénykeverővel/ Premiešať pomocou prístroja Vortex/ Promíchat pomocí přístroje Vortex/ Разбъркване на епруветките с миксер Vortex/ Segada torukesi Vortexi mikseriga/ Αναμίξτε τους σωληνίσκους με αναδευτήρα Vortex/ Amestecaţi eprubetele cu ajutorul unui agitator vortex/ Mešanje cevčic z mešalnikom Vortex/ Sekoita putket Vortex sekoittajalla
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/ kalibrátor X/ kalibrátor X/ kalibrátor X/ калибратор X/ kalibraator X/ Βαθμονομητής X/ calibrator X/ kalibrator X/ kalibraattori X
CTR1 / CTR2	Control X/ Kontrolle X/ Contôle X/ controllo X/ control X/ Controle X/ controle X/ Kontrol X/ Kontroll X/ kontrolne X/ Ellenőrző X/ Kontrolné X/ Kontrolní X/ Контролен X/ Kontroll X/ ελέγχου X/ control X/ Kontrolni X/ Kontrolli X
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
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

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.

Table of Content

Instructions for use

hGH ELISA E02	96 Determinations
Principle of the test	Sandwich ELISA
Duration (incubation period)	2.25 h
Antibody Conjugate	ready for use
Enzyme Conjugate	ready for use
Buffer and Substrate	ready for use
Reference material	2. International Standard WHO/ NIBSC 98/574, recombinant hGH
Calibrators	5 single calibrators **1 ng/mL – 25 ng/mL**, ready for use, recombinant hGH
Assay Range	0.25 ng/mL - 25 ng/mL
Controls	2 Controls, ready for use
Sample	human serum / plasma
Required sample volume	20 µL for single determination, 40 µL for double
Sample dilution	undiluted
Analytical sensitivity	ø 0.25 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 ELISA E02 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: **Controls CTR1 and CTR2**
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 Calibrators 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.

The E02 is also suitable e.g. for measurements in cell cultures or in non-serum samples (e.g., hGH urine). If required, the Mediagnost ELISA hGH SENSITIV E022 is available for highly sensitive measurements

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:

20 µL for single determination, 40 µL for double determination

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 200 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

- If no extreme values are to be expected, the use of **20 µL** undiluted serum or plasma sample is optimal.
- If necessary, samples can be diluted in Dilution Buffer **DIL**.

- In the other samples than serum or 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 calibrator curve.

MTP	**Microtiter plate,** ready for use, coated with rabbit-anti-hGH-antibody. Wells are separately breakable.	**(8x12) wells**
CAL A-E	**Calibrators**, ready for use, (recombinant human hGH), concentrations are given on vial labels and on quality certificate.	**5 x 750 µL**
CTR1	**Control 1,** ready for use, (human serum), concentration is given on quality certificate.	**1 x 750 µL**
CTR2	**Control 2,** ready for use, (human serum), concentration is given on quality certificate.	**1 x 750 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 30 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 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 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.

Dilution of reagents

The dilution of the **Controls CTR1** and **CTR2** should be according to the dilution of the respected samples, **generally undiluted 20 µL/well.**

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**, Controls **CTR1 and CTR2** 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**, Controls **CTR1**, **CTR2** 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

NOTES: Bring all reagents to **room temperature** +**20-25°C** before use
All determinations (**Calibrators**, **Controls** and **samples**) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended. The assay must be performed at room temperature.

Please pipette on before in all needed wells 100 µL of Dilution Buffer DIL.

1) Add additional **20 µL Dilution Buffer DIL** in wells A1/A2 (blank).
2) Pipette in positions B1/2 **20 µL of Calibrator A (1 ng/ml)**

   Pipette in positions C1/2 **20 µL Calibrator B (5 ng/ml)**,

   Pipette in positions D1/2 **20 µL Calibrator C (10 ng/ml)**,

   Pipette in positions E1/2 **20 µL Calibrator D (15 ng/ml)**,

   Pipette in positions F1/2 **20 µL Calibrator E (25 ng/ml)**.

   To control the correct accomplishment **20 µL** of the undiluted (or in respective dilution rate of the sample) **Controls CTR1** and **CTR2** can be pipetted in positions G1/2 and H1/H2.

   Pipette **20 µL of the undiluted sample** in the rest of the wells, according to requirements.
3) Cover the wells with sealing tape and incubate the plate for **1 hour** at **room temperature** (shake at 350 rpm).
4) After incubation aspirate the contents of the wells and wash the wells **3 times** with **300 µL Washing Buffer WB** / well.
5) Following the last washing step, pipette **100 µL** of the **Antibody Conjugate DET** in each well, cover the wells with the sealing tape and **incubate 30 minutes** (shake at 350 rpm).
6) Subsequently –**without a washing step!** - pipette **100 µL** of the **Enzyme Conjugate EC** in each well, cover the wells with the sealing tape and incubate additional **30 minutes without shaking**.

The solutions should now be mixed through the addition; slight shaking or tapping on the border of the microtiter plate could support this. Attention: The risk of the cross contamination is increased through the high filled volume of the wells.

7) After incubation, wash the wells **3 times** with **Washing Buffer WB** as described in step 4.
8) Pipette **100 µL** of the **Substrate S** in each well. Incubate the plate for **15 minutes** in the dark at **room temperature**.
9) Stop the reaction by adding **100 µL** of **Stop Solution STP**.

   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	**1**	**5**	**10**	**15**	**25**
µIU/mL	3	15	30	45	75

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, Controls 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 µIU/mL, according the chosen unit for the Calibrators) of the samples and controls CTR1 and CTR2 is calculated automatically by your program.

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.

	Blank	**A**	**B**	**C**	**D**	**E**
ng/mL	**0**	**1**	**5**	**10**	**15**	**25**
$OD_{(450\text{-}620\ nm)}$	0.0658	0.152	0.717	1.303	1.659	2.428

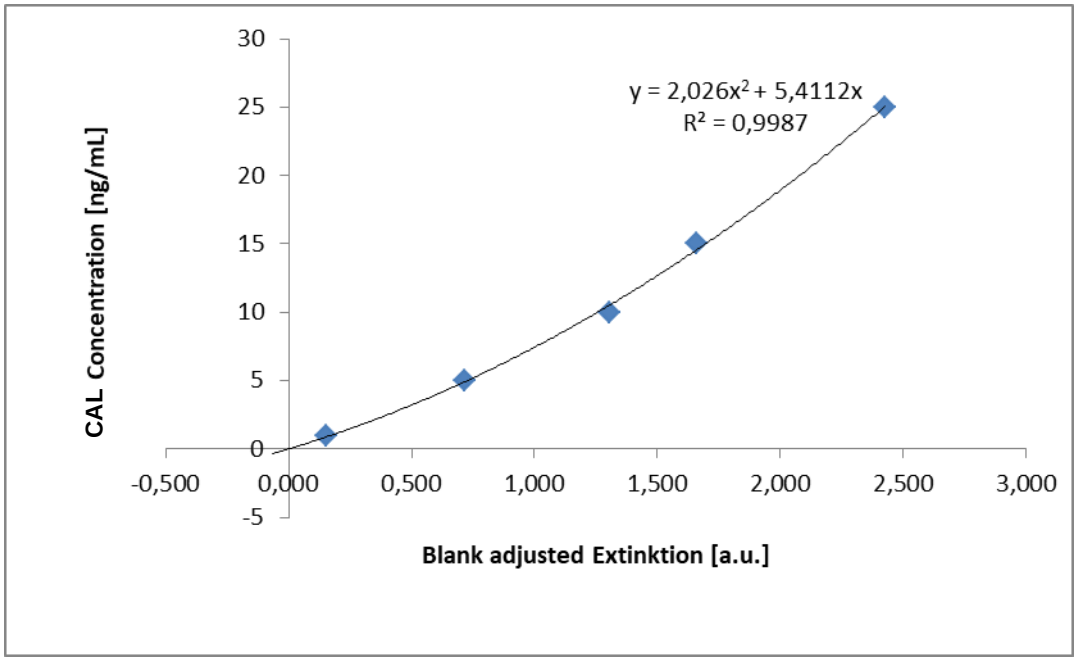

Figure 1: Exemplary calibration curve

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

Measured extinction of your sample 1.3685
Measured extinction of the blank 0.0658

Your measurement program will calculate the hGH concentration of the sample automatically by using the difference of sample and blank for the calculation. You only have to determine the most suitable curve fit.
In this exemplary case the following equation is solved by the program to calculate the hGH concentration in the sample:

$1.3027 = 2.026\ x^2 + 5.4112x$
10.487=ng/mL

as the sample is undiluted the hGH concentration of the sample is 10.487 ng/mL.

10 LIMITATION OF PROCEDURE

The Mediagnost human Growth-Hormone ELISA, E02 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.

11 EXEMPLARY VALUES

We investigated hGH serum concentrations of 104 healthy blood donors in the age of 18-69 years.

Table 1 Exemplary values hGH serum concentrations 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

12 PERFORMANCE CHARACTERISTICS

12.1 Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the E02 is ø 0.25 µg/L.

12.2 Specificity

Here the results of the evaluation of Mediagnost E022 can be transferred, because the same antibodies are used and thus they should show the same 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 different concentrations. Here no significant influence of Somavert was detected (Table 2). But in E022 sensitive Growth Hormone ELISA [9] pegvisomant (100 mg/L) has been added to hGH enriched samples containing 2.6 and 10.1 µg/L hGH and measured hGH concentrations increased to 154% and 108% respectively.

Table 2 Cross reactivity to Pegvisomant (trade name Somavert)

Somavert [mg/L] In Dilution Buffer DIL	**Concentration measured in E02 [mg/L]**	**% Cross-reactivity**
100	0.0063	0.0063
10	0.0047	0.047
1	0.0027	0.27
0.1	0.00086	0.86
0.01	0.0002	2

12.3 Precision

Intra-Assay-Variation

Two samples have been measured 16 times in the same assay. The results are shown in table 3. The measured coefficient of variation (CV) is 4.19% on average.

Table 3 Intra-Assay Variation

	Number of determinations	**Mean value (µg/L)**	**Standard deviation (µg/L)**	**VC (%)**
Sample 1	16	5.84	0.27	4.70
Sample 2	16	14.92	0.55	3.68

Inter-Assay Variance

Serum samples where measured in independent assays. On average the coefficient of variation was 5.89%. Results are shown in detail in table 4.

Table 4 Inter-Assay Variation

	Number of single determinations	Mean value (µg/L)	Standard deviation (µg/L)	VC (%)
Sample 1	10	2.57	0.15	5.68
Sample 2	10	5.80	0.31	5.38
Sample 3	10	10.86	0.72	6.62

12.4 Linearity

Linearity of the E02 was tested by dilution of different serum samples and recalculation of the measured hGH concentration. Results are shown in Figure 2. Samples can be diluted in a broad range according to the requirements of the experimental setting (e.g. baseline hGH, stimulation assays).Here samples can be used undiluted.

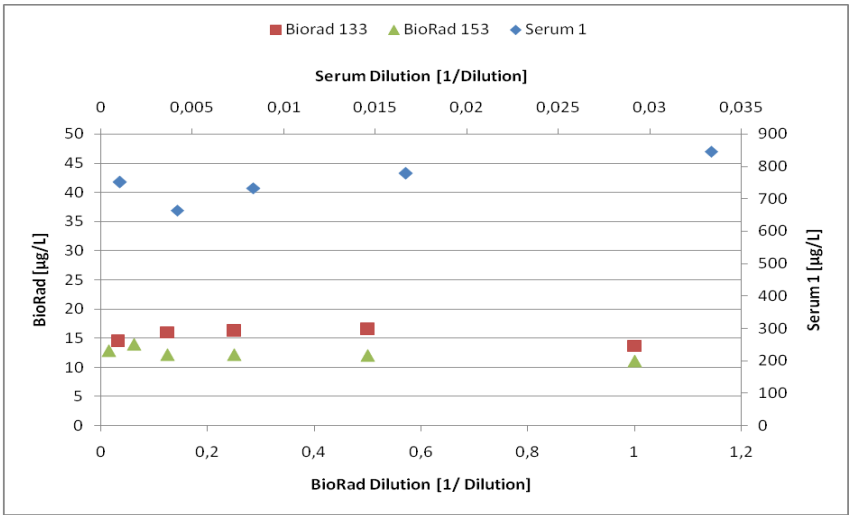

Figure 2 Linearity of sample dilution. Three samples with different amount of hGH were diluted and hGH concentration was recalculated. Secondary axis was used for Serum 1 because of extreme high hGH concentration.

12.5 Recovery and Accuracy

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

Table 5 Recovery of recombinant human GH in Serum.

Sample	endogenous hGH [µg/L]	enriched 10 µg/L	relative Recovery [%]
Serum 1	0.38	9.53	96.5
Serum 2	1.05	9.09	92.0
Urine	0.11	9.03	91.4

12.6 Interference

The antibodies used in E022 and E02 are identical, thus data regarding interference can be applied to the E02, too.
Interference of bilirubin and triglycerides has been tested by Langkamp et al in E022 and results are published in [9]. Here neither bilirubin (up to 200 mg/L) nor triglycerides (up to 200 mg/mL) showed a significant interference with hGH measurement. 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%).

13 ASSAY COMPARISON

To demonstrate identity of E02 and E022 hGH concentrations of fifteen serum samples have been measured by both assays. The linear regression analysis reveals a coefficient of correlation of 0.997, which demonstrates an excellent correlation between both assays and a slope of 1.056 proves a high agreement of absolute measured concentrations (see Figure 3). Thus, it is possible to transfer the results of the E022 validation to the E02, including the cut-off values and expected values in healthy humans.

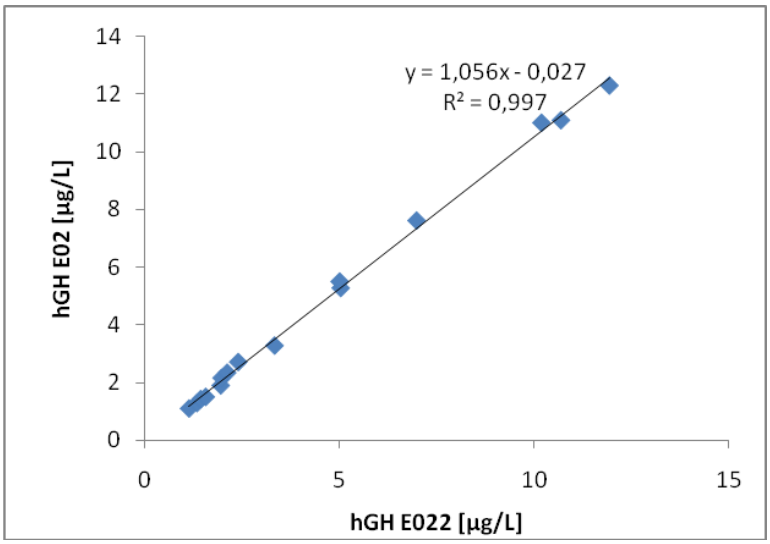

Figure 3 Assay comparisons. Fifteen serum samples were measured in different lots of E02 and E022 mean values are shown here in comparison.

13.1 Species Cross-Reactivity

Several commercially available animal sera have been used as samples in this assay. NO signal was detected in serum of the following species:

Horse, Rabbit, Dog, Guinea pig, Sheep, Goat, Cat, Pig, Chicken, Cow

A small signal can be detected if another dilution is used (e.g. 1:5), whether this signal is hGH specific and 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.

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15 INTERNATIONAL ASSAY DESCRIPTION

International Test Description

CAL A-E			
CTR1			
CTR2			
DET			
EC			
WB	**WB 20x**	**1:20 A. dest.**	**➔ WB 1:20**
SPE			
°C 20-25°C			
100 µL	**DIL**		A1 - End
20 µL	**DIL**		A1/A2
20 µL	**CAL A**	**(1 ng/mL)**	B1/B2
20 µL	**CAL B**	**(5 ng/mL)**	C1/C2
20 µL	**CAL C**	**(10 ng/mL)**	D1/D2
20 µL	**CAL D**	**(15 ng/mL)**	E1/E2
20 µL	**CAL E**	**(25 ng/mL)**	F1/F2
20 µL	**CTR1**		G1/G2
20 µL	**CTR2**		H1/H2
20µL	**SPE**		
TAPE			
1 h °C 20-25°C ↔ 350 rpm			
3x 300 µL	**3x 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			
3x 300 µL	**3x WB 1:20**		
100 µL	**S**		
0.25 h °C 20-25°C			
STP			
MEASURE			

16 ASSAY PROCEDURE

Preparation of reagents		
CAL A-E **CTR1** **CTR2** **DET** **EC**	**Calibrators** (each 750 µL) **Control 1** (750 µL) **Control 2** (750 µL) **Antibody Conjugate** (12 mL) **Enzyme Conjugate** (12 mL)	Bring the ready for use reagents to room temperature: **20°-25°C**
WB	**Washing Buffer Conc.**	Dilute **1:20** with **Aqua dest.** ➔ **WB 1:20**
Dilution of samples is generally not necessary; just use **20 µL** per single determination.		
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**	Pipette in all **required number of wells**
20 µL	Dilution Buffer **DIL** (**Blank**)	A1/A2
20 µL	Calibrator **A** (**1 ng/mL**)	B1/B2
20 µL	Calibrator **B** (**5 ng/mL**)	C1/C2
20 µL	Calibrator **C** (**10 ng/mL**)	D1/D2
20 µL	Calibrator **D** (**15 ng/mL**)	E1/E2
20 µL	Calibrator **E** (**25 ng/mL**)	F1/F2
20 µL	Control **CTR1**	G1/G2
20 µL	Control **CTR2**	H1/H2
20 µL	Sample **SPE**	Pipette sample in the rest of the wells according the requirements.
Cover the wells with the sealing tape.		
Sample Incubation: **1 h at 20-25°C**, **350 rpm**		
3 x 300 µL	Aspirate the contents of the wells and **wash** 3 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		
3 x 300 µL	Aspirate the contents of the wells and **wash 3 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.		