

# hGH - SENSITIVE ELISA

(Human Growth Hormone; Somatropin)

Enzyme Immunoassay for the high-sensitive Quantitative  
Determination of  
**Human Growth Hormone**

distributed in the US/Canada by:  
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REF **E022**

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





of IFY is to be used only for reference purposes. Contents especially technical, methodical and regulations data, may not be amended.

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# SYMBOLS

according to DIN EN ISO 15223-1

	Expiry date
	Consider instructions for use
	In vitro diagnostic medical device. For In Vitro Diagnostic Use.
	Lot-Batch
	Manufactured by
	Catalogue number
	Store at between
	Contains sufficient for x tests
	Keep away from sunlight
<b>MTP</b>	Mikrotiter plate

## INTENDED USE

An enzyme immunoassay for the quantitative *in-vitro diagnostic* measurement of levels of human growth hormone in serum and plasma. Human growth hormone measurements are used in the diagnosis and treatment of disorders involving the anterior lobe of the pituitary gland.

## SUMMARY AND EXPLANATION

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 gene expression results in a non-glycosylated 22 kDa protein consisting of 191 amino acids. The other 20% of gene expression results in a variant form of 20 kDa by alternative splicing. Additionally, several smaller variants can be found in circulation as well as translational modified proteins and different degrees of protein aggregation. Further on, 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 attractions as physiologic stress or hypoglycaemia and amino acids result in additional hGH secretion, induced by the hypothalamic hormones Somatostatin and GH-Releasing Hormone (GHRH). Age, sexual steroids, nutritional status, illness and emotions influence the amount of secreted hGH. Because of the multitude of influencing factors the normal quantitative secretion is not known.

Physiological functions of hGH are also manifold. These functions are partially exerted by Insulin-like Growth Factors (IGFs). In children and adolescent the hGH system is the main regulator of growth. If the hGH system fails totally, human growth will end at 120 cm. Beside regulation of growth hGH exerts an anabolic effect on muscle and connective tissue as well as on bone and different other organs (heart, intestine). Further hGH was proved to have a lipolytic effect.

Growth Hormone pathology is characterized by extreme high or extreme low hGH secretion. During childhood it is the Growth Hormone deficiency congenital or acquired, which leads to microsomia. For diagnosis of Growth Hormone deficiency an hGH stimulation test has to be done or the spontaneous excretion must be investigated. The therapy consists of substitution of endogenous Growth Hormone by recombinant hGH resulting in normalization of growth. In adulthood hGH deficiency is mostly caused by pituitary adenoma (and their surgical excision). hGH deficiency shows typical disease pattern, equivalent to advanced aging (adipositas, muscle dystrophy, arteriosclerosis, osteoporosis, adynamia). Substitutional therapy is a well-known, approved and efficient therapy of severe Growth Hormone deficiency in adulthood. Therapeutic success is directly as well as indirectly proved by measurement of IGF in serum.

Excessive hGH secretion, mostly caused by pituitary adenoma, results in childhood in gigantism, in adulthood in acromegaly, leading to enlarged extremities, diabetes, heart insufficiency and tumor growth. Surgical excision of the adenoma is the therapy of choice. If tumor excision is not possible or incomplete, a medicinal therapy with somatostatin preparation will be conducted, resulting in inhibition of hGH production. Alternatively hGH analoga (e.g. Pegvisomant) are used to block the hGH receptor and thereby inhibit action of endogenous hGH.

Determination of human Growth Hormone (hGH, Somatropin) is done for diagnosis of Growth Hormone deficiency or Growth Hormone excess (arcomegaly). During medicinal and/or after surgical therapy of arcomegaly Growth Hormone (and IGF-I) measurement is used for therapy control.

## **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 antiserum in turn binds hGH. After washing, a streptavidin-peroxidase-enzyme conjugate will be added, which will bind highly specific to the biotin of the antiserum and will catalyze the substrate to change the color quantitatively depending on the hGH level of the sample.

## **WARNINGS AND PRECAUTIONS**

1. For In Vitro Diagnostic Use.
2. For professional use only.
3. Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements.
4. Do not use obvious damaged or microbial contaminated or spilled material.
5. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. Follow strictly the test protocol. 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.

6. Before use, all kit components should be brought to room temperature at 20 - 25°C (68 - 77°F). Precipitates in buffers should be dissolved before use by thorough mixing and warming. Temperature WILL affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
7. Do not mix reagents of different lots. Do not use expired reagents.
8. The microtiter plate contains snap-off strips with single breakable wells. The frame provided must be used.
9. Caution: Following components contain human serum: Control Serum KS. 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) antibodies. No known methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.
10. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
11. Use separate pipette tips for each sample, control and reagent to avoid cross contamination.
12. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
13. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
14. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

### Reagents AK, EK, VP, WP

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 regional/ /national/ international regulations.	local/

### 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 Stop solution contains 0.2 M acid sulphur acid (H<sub>2</sub>SO<sub>4</sub>)

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.

## MATERIALS PROVIDED

- 1) **Microtiter plate**, ready for use: Microtiter plate with 96 wells, divided into 12 strips with 8 break-apart wells coated with anti-human Growth Hormone Antibody.
- 2) **Standards A-E**, lyophilized: Contain recombinant hGH (NIBSC 98/574). Standard values are between 0.05 - 1 ng/mL (50, 150, 300, 600 and 1000 pg/mL) hGH. Each vial must be reconstituted with **750 µL of Dilution Buffer VP**.
- 3) **Dilution Buffer VP, 120 mL**, ready for use, please use for the reconstitution of **Standards A – E** and of **Control Serum KS** as well as for the **sample** and **Control Serum KS** dilution.
- 4) **Control Serum KS**, lyophilized, contains human serum and must be reconstituted with **500 µL Dilution Buffer VP**. The hGH target value concentration and the respective range are given on the certificate. The dilution of the **Control Serum KS** should be according to the dilution of the samples.
- 5) **Antibody Conjugate AK, 12 mL**, ready-made solution, contains rabbit biotinylated anti-hGH antibody.
- 6) **Enzyme Conjugate EK, 12 mL**, ready-made solution, contains HRP (Horseradish-Peroxidase)-labelled Streptavidin.
- 7) **Washing Buffer WP, 50 mL**, 20 X concentrated solution.
- 8) **Substrate S, 12 mL**, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H<sub>2</sub>O<sub>2</sub>-Tetramethylbencidine.
- 9) **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

1. Precision pipettes and multichannel pipettes with disposable plastic tips
2. Graduated cylinder for diluting **Washing Buffer WP**
3. Distilled or deionized water for dilution of the **Washing Buffer WP, 950 mL**
4. Vortex-mixer
5. Microtiter plate shaker (350 rpm)
6. Microtiter plate washer (recommended)
7. Micro plate reader ("ELISA-Reader") with filter for 450 and  $\geq 590$  nm.
8. Polyethylene PE/Polypropylene PP tubes for dilution of samples

## TECHNICAL NOTES

Bring all reagents to room temperature **20 - 25°C** (68 - 77°F) before use.

**Washing Buffer WP**: has to be diluted 1:20 with distilled/deionized water before use (e.g. add the complete contents of the (20x) concentrated **Washing Buffer WP** (50 mL) into a graduated flask and fill to a final volume of 1000 mL). Attention: After dilution the **Washing Buffer WP** is only 4 weeks stable at 2-8°C (35.6-46.4°F) dilute only according to requirements.

The **Dilution Buffer VP** must be used for the **reconstitution** of the lyophilized components **Standards A – E** and **Control Serum KS**. Each bottle of **Standards** must be reconstituted with **750 µL Dilution Buffer VP**. **Control Serum KS** must be reconstituted with **500 µL of Dilution Buffer VP**. 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.

### Incubation

**Incubation at room temperature means: Incubation at 20–25°C (68–77°F)**. The **Substrate Solution S**, stabilised H<sub>2</sub>O<sub>2</sub>-Tetramethylbencidine, is photosensitive—store and incubation in the dark.

### Shaking

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.

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

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

## Storage Conditions

Store the kit at 2-8°C (35.6-46.4°F) after receipt until its expiry date. The lyophilized reagents should be stored at -20°C (-4°F) after reconstitution. Avoid repeated thawing and freezing.

The **Substrate Solution S**, stabilised H<sub>2</sub>O<sub>2</sub>-Tetramethylbencidine, is photosensitive – store and incubate in the dark at 2-8°C (35.6-46.4°F).

## 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 (35.6-46.4°F) in the clip-lock bag, use in the frame provided. The **reconstituted components: Standards A-E and Control Serum KS** must be stored at -20°C (-4°F) (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. In case you plan to perform multiple independent hGH determinations with one kit, you should aliquot the components prior to freezing into suitable smaller volumes.

The 1:20 diluted **Washing Buffer WP** is 4 weeks stable at 2-8°C (35.6-46.4°F).

## Preparation of reagents

Bring all reagents to room temperature 20 - 25°C, (68 - 77°F) 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.

## Reconstitution



The **Standards A – E** and **Control KS** are reconstituted with the **Dilution Buffer VP**. 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 Serum KS** with the **Dilution Buffer VP** in the same ratio (1:26) as the sample.

The required volume of **Washing Buffer WP** is prepared by 1:20 dilution of the provided 20fold concentrate with Aqua dest.

### **SPECIMEN COLLECTION, PREPARATION AND STORAGE**

Human GH is secreted pulsatile during the day/night. Therefore, in clinical application stimulation test are used to measure peak GH concentrations.

Serum as well as plasma samples are suitable (significant deviation of hGH levels in corresponding Serum, Heparin-, or EDTA-Plasma samples were not found). Common cell culture medium was found to be suitable. An external sample preparation prior to assay is not required (see below).

Samples should be handled as recommended in general: collected and refrigerated as fast as possible. In case there will be a longer period (>24 hours) between the sample withdrawal and determination, store the undiluted samples frozen at  $-20^{\circ}\text{C}$  ( $-4^{\circ}\text{F}$ ) in tightly closable plastic tubes. Avoid repeated freeze-thaw cycles of serum/plasma (if required, please sub-aliquot) although hGH levels were found to be unaffected by a few cycles, (5x) in our experiments.

The high sensitivity of the assay allows hGH measurement of hGH in small sample volumes, which is limited by pipetting accuracy rather than the amount of hGH. In most determinations (serum or plasma samples, and no extreme values expected) a **dilution from 1:10 to 1:50 with Dilution Buffer VP** should be suitable. According to expected hGH levels, the dilution with Buffer can be higher or lower. In general, a dilution of **1:26** for serum- or plasma samples is appropriate. The hGH concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatants.

#### Suggestion for dilution protocol:

Pipette 250  $\mu\text{L}$  **Dilution Buffer VP** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series); add 10  $\mu\text{L}$  serum- or plasma samples (dilution 1:26). After mixing use 2 x 100  $\mu\text{L}$  of this dilution in the assay.

### **ASSAY PROCEDURE**

#### **Assay Procedure**

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

When performing the assay **Blank, Standards A-E, Control KS** 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 AK** and 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 **Substrate Solution S**.

- 1) Add **100  $\mu\text{L}$  Dilution Buffer VP** in wells **A1/A2** (blank).
- 2) Pipette in positions **B1/2 100  $\mu\text{L}$  Standard A (0.05 ng/mL)**  
Pipette in positions **C1/2 100  $\mu\text{L}$  Standard B (0.15 ng/mL)**  
Pipette in positions **D1/2 100  $\mu\text{L}$  Standard C (0.3 ng/mL)**  
Pipette in positions **E1/2 100  $\mu\text{L}$  Standard D (0.6 ng/mL)**  
Pipette in positions **F1/2 100  $\mu\text{L}$  Standard E (1 ng/mL)**

For control purpose pipette 100 µL of the 1:26 (or respective dilution as the sample) diluted Control Serum 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 (68 - 77°F)** (shake at 350 rpm).
- 4) After incubation aspirate the contents of the wells and wash the wells 5 times with **300 µL Washing Buffer WP**/ 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 AK** 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 EK** in each well and incubate additional **30 minutes without shaking**.
- 8) After incubation wash the wells **5 times** with **Washing Buffer WP** as described in step 4.
- 9) Pipette **100 µL of the TMB Substrate Solution S** in each well.
- 10) Incubate the plate for **15 minutes in the dark at room temperature 20 - 25°C (68 - 77°F)**.
- 11) Stop the reaction by adding **100 µL of Stopping Solution SL**.
- 12) Measure the colour reaction within 30 minutes at **450 nm (reference filter ≥590 nm)**.

## QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state or local standards/laws. All standards and kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

## CALCULATION OF RESULTS

### Establishing the Standard Curve

The 2nd International Standard for hGH, NIBSC Code 98/574 (6), was used as standard material. The International Standard 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 standard material is recommended in line with the current standardisation efforts for hGH Immunoassays (7,8).

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 standard E should be above 1.00. Samples, which yield higher absorbance values than **Standard E**, are beyond the standard curve. For reliable determinations these samples should be re-tested with a higher dilution.

The standards provided contain the following concentration of hGH:

<b>Standard</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>
ng/mL	0.05	0.15	0.30	0.60	1.0
pg/mL	50	150	300	600	1000

$\mu\text{IU/mL}$	0.15	0.45	0.9	1.8	3.0
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- 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 absorbencies of all samples and standards.
- 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 hGH concentration in ng/mL (or pg/mL, or  $\mu\text{IU/mL}$ , according the chosen unit for the standards) of the samples can be calculated by multiplication with the respective dilution factor.

### Example of Typical Standard Curve

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

Standard	A	B	C	D	E
ng/mL	0.05	0.15	0.30	0.60	1.0
Extinction	0.19	0.445	0.845	1.48	2.245

### INTERPRETATION OF RESULTS

The test results should not be the only base for therapeutic decisions. The results should be interpreted in regard to anamnesis, further clinical observations and results of other diagnostic investigations. Further, each laboratory has to establish own reference and cut-off values corresponding to the relevant group of patients.

Usually, the maximal peak of growth hormone secretion is measured in at least 2 independent stimulation assays (e.g. insulin or arginine stimulation). If the used test system is calibrated to WHO standard 98/574, as Mediagnost E022 is, a secretion peak of less than **8 ng/mL** indicates a possible growth hormone deficiency. But as growth hormone secretion is continuous between normal and pathological any cut-off is only a non-binding benchmark.

We recommend to take the international and national guidelines for diagnosis and treatment of growth hormone deficiency / acromegaly into account.

### EXPECTED NORMAL VALUES

As growth hormone is secreted pulsatile mainly enduring the night sleep valid normal values can hardly be determined. Standard procedures are arginine or insulin stimulation tests, after injection of stimulating substance growth hormone concentration is measured over a period of time. We investigated hGH serum concentration of 104 healthy blood donors in the age of 18-69 years without any stimulation.

	<b>female</b>	<b>male</b>
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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

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests. Furthermore, we recommend that each laboratory determine its own range for the population tested.

## LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION, PREPARATION AND STORAGE for details.

Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as rinsing the test strips and thorough mixing of all prepared solutions.

Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled evenly with Washing Solution, and that there are no residues in the wells.

Instructions for using appropriate photometers are to be observed; check adjustment of proper wavelength and reference wavelength respectively.

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 and pharmaceutical substances has been tested for the indicated concentrations. Higher concentrations or other substances may interfere with the measurement.

If a cut-off of **8 µg/L** is used in the growth hormone stimulation test for diagnosis of growth hormone deficiency, literature states a diagnostic sensitivity and specificity about 80%, if all clinical preconditions for testing are fulfilled [10]

## PERFORMANCE CHARACTERISTICS

### Sensitivity

The analytical sensitivity of the hGH SENSITIVE ELISA E022 yields 0.0115 ng/mL (equal to 11.5 pg/mL, equal to 1.15 pg per well; 2x SD of zero standards in 16-fold determination).

### Specificity

The only human protein with significant sequence similarities to growth hormone is prolactin. Testing a 200 ng/mL prolactin solution in this assay, no cross reactivity was detected.

Further, Pegvisomant (trade name Somavert) a growth hormone analogue and pharmaceutical used in acromegaly therapy has been tested for cross reactivity in different concentrations. Here no significant influence of Somavert was detected (Table 1). But in Growth Horm IGF Res 18 (2008) 526-532 the authors added 100 mg/L pegvisomant to hGH enriched samples containing 2.6 and 10.1 µg/L hGH and measurement results were 154% and 108% of the expected values respectively [9].

**Table 1:** Cross reactivity to Somavert in assay buffer

Somavert [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

**Reproducibility****Intra Assay Variance**

Intra assay variance was measured by applying a sample 16 times in one assay. The results are shown in Table 2. 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 2:** 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**

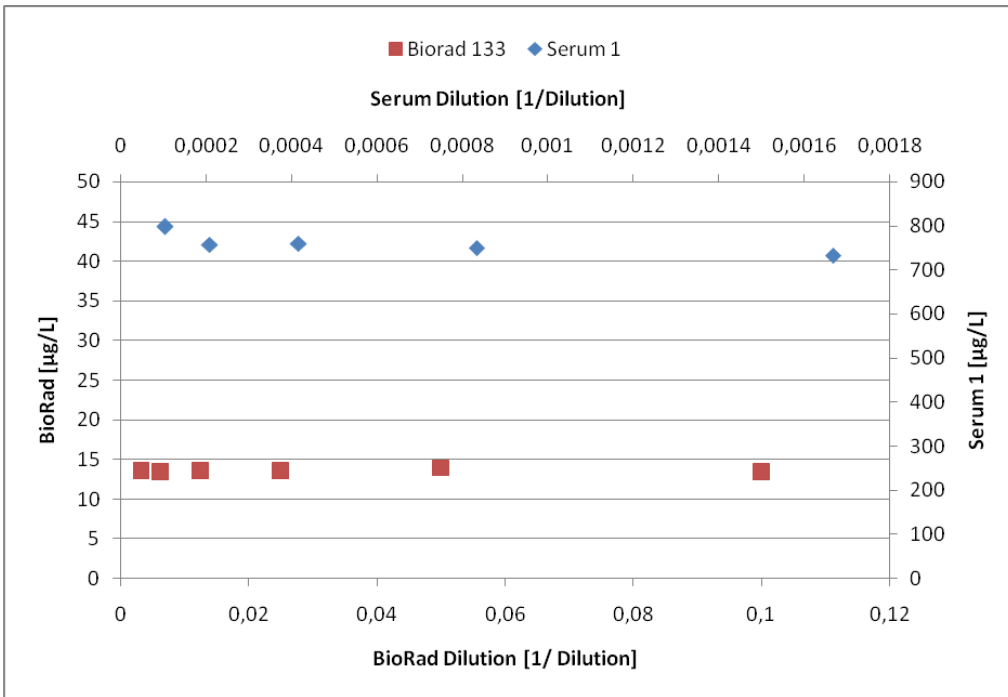
Serum samples were measured in independent assays. On average the coefficient of variation was 4.34%. Results are shown in detail in table 3. Here also externally acquired data are available: The mean coefficient of variation for inter-assay variance at 2.39; 5.37 and 14.33 µg/L hGH was 5.98%; 3.93% and 3.12%, respectively [9].

**Table 3:** 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

**Linearity**

Linearity of the E022 was tested by dilution of different serum samples and recalculation of the measured hGH concentration. Results are shown in Figure 1. Samples can be diluted in a broad range according the requirements of the experimental setting (e.g. baseline hGH, stimulation assays). We recommend a standard dilution of 1:26.



**Figure 1:** Linearity of sample dilution. Two 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.

## Interference

Interference of bilirubin and triglycerides has been tested by Langkamp et al and results are published in Growth Horm IGF Res 18 (2008) 526-532 [9]. Here neither bilirubin (up to 200 mg/L) nor triglycerides (up to 200 g/L) 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%) (Table 4). Table 5 summarizes the results of corresponding investigations done by Mediagnost.

**Table 4:** 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 5:** 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

## 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 buffer calculated. Results are shown in table 6.

**Table 6:** Recovery of recombinant human GH in Serum

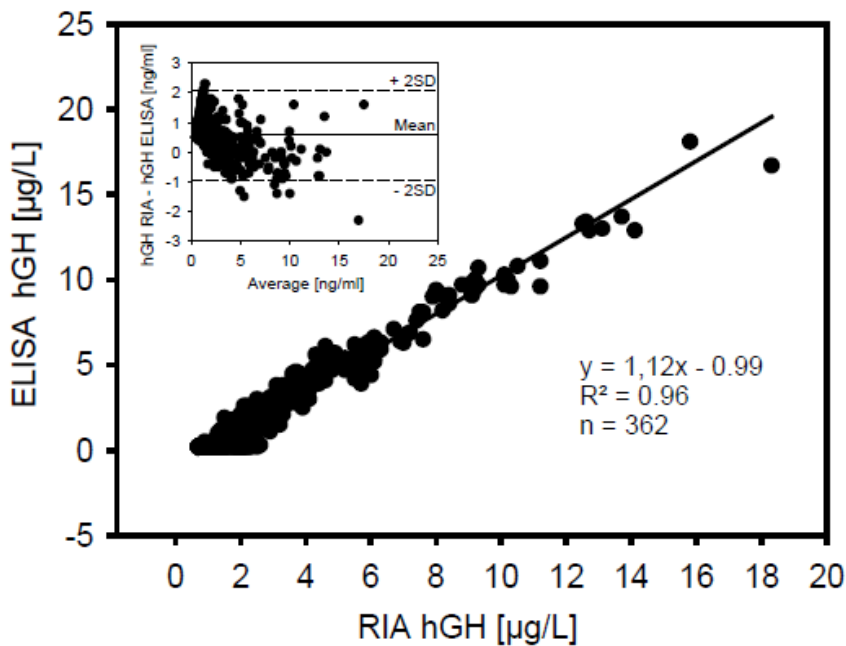
NIBSC Rec. hGH	VP		Serum 1 PAA539		Serum 2 PAA 574	
	ng/mL	%	ng/mL	%	ng/mL	%
20	19.34	96.7	17.51	83.2	17.53	81.9
10	9.81	98.1	9.28	84.0	9.18	80.4
5	5.34	106.7	4.99	82.67	5.9	92.0
0	0	-	1.04	-	1.41	-

## COMPARISON STUDIES

### Assay Comparison

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 2).



**Figure 2:** Assay Comparison



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## SUMMARY – Mediagnost hGH-sensitive ELISA E022

Reagents:	Reconstitution:	Dilution/ Mixing:
Standards A-E	in 750 µL Dilution Buffer	
Control Serum KS	in 500 µL Dilution Buffer VP	<b>1:26 with Dilution Buffer VP</b>
Washing Buffer WP		<b>1:20 with Aqua. dest.</b> (e.g. add the complete contents of the flask (50 mL) into a graduated flask and fill with A.dest. to 1000 mL).
<b>Sample dilution:</b> with <b>Dilution Buffer VP</b> generally <b>dilution of 1:26</b> (e.g. dilute 10 µL serum with 250 µL Dilution Buffer VP). Use <b>100 µL</b> per determination.		
Before assay procedure bring all <b>reagents</b> to <b>room temperature</b> .		

### Proposal of Assay Procedure for double determinations

Pipette	Reagents	Position
100 µL	Dilution Buffer <b>VP (blank)</b>	A1/2
100 µL	Standard <b>A (0.05 ng/mL)</b>	B1/2
100 µL	Standard <b>B (0.15 ng/mL)</b>	C1/2
100 µL	Standard <b>C (0.30 ng/mL)</b>	D1/2
100 µL	Standard <b>D (0.6 ng/mL)</b>	E1/2
100 µL	Standard <b>E (1.0 ng/mL)</b>	F1/2
100 µL	Control Serum <b>KS</b> (1:26 diluted)	G1/2
100 µL	Sample (1:26 diluted)	Pipette sample in the rest of the wells according the requirements
Cover the wells with the sealing tape.		

### Sample Incubation: 2 h at RT, 350 rpm

5 x 300 µL	Aspirate the contents of the wells and <b>wash</b> 5x with 300 µL each Washing Buffer <b>WP/well</b>	in each well
100 µL	<b>Antibody Conjugate AK</b>	in each well

### AK Incubation 0.5 h at RT, 350 rpm

100 µL	Enzyme conjugate <b>EK, without washing the wells (!)</b> – add to the previously pipetted AK-solution <b>thereto</b> , thereby simultaneously mixing or mix shortly through cautious tapping on the MTP. <b>Attention: high filled volume of the wells!</b>	in each well
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### EK Incubation 0.5 h at RT, without

5 x 300 µL	Aspirate the contents of the wells and <b>wash</b> 5 x with 300 µL each Washing Buffer <b>WP/well</b>	in each well
100 µL	Substrate Solution <b>S</b>	in each well

### Substrate S Incubation: 15 min. in the dark at RT

100 µL	Stopping Solution <b>SL</b>	in each well
Measure the absorbance within <b>30 min</b> at <b>450 nm</b> with <b>≥ 590 nm</b> as reference wavelength.		

