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Research
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Human GH ELISA (Growth Hormone; Somatotropin)

Cat. No.: RMEE02R

CLINICAL RELEVANCE

Determination of human Growth Hormone (hGH, Somatropin) is done for diagnostic 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.

TECHNICAL PROPORTIES AND APPLICATIONS

The hGH ELISA RMEE02R –

- is well suited for determination of hGH in **serum** and **plasma** (native samples, hGH profiling, samples of stimulation and suppression tests)
- is calibrated against the **reference assay** of the KIGS/IGLU study, in house-RIA of University Children Hospital Tübingen (see literature 1)
- due to its high **sensitivity** (0.25 ng/ml ≅ 250 pg/ml) it is also very well suited to measure hGH in cell culture supernatants and other non-serum samples (e.g. hGH urine – excretion test), If required, a ELISA hGH Sensitive RMEE022 is available for high sensitive determinations. (Sensitivity 0.0016 ng/ml ≈ 1.6 pg/ml)
- is **highly specific** and therefore allows measurement of hGH during simultaneous application of hGH and hGH-analoga e.g. Pegvisomant (e.g. Somavert® of the Pfizer Inc.)
- is **fast**: total incubation time is only 2.25 hours and applies high affine polyclonal antibodies against 22 kDa recombinant hGH
- is calibrated against recombinant hGH (2nd International Standard, **NIBSC 98/574**; see literature 6)
  Single Standards of **1, 5, 10, 15 and 25 ng/ml hGH** are supplied within our kit, control serum consisting of human serum is also provided, microtiterplate is breakable to single wells

INTRODUCTION

(See references 2-5)

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-glycolsylated 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 more smaller variants can be found in circulation as well as translational modificated 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. GH is species specific.

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 wells 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. Therapeutical success is directly as well as indirectly proved by measurement of IGF in serum.

Excessive hGH secretion, mostly causes by pituitary adenoma, results in childhood in gigantism, in adulthood in acromegalie, 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.

METHODOLOGY

Assay Characteristics

The hGH ELISA RMEE02R 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 binds in turn hGH. After washing, a streptavidin-peroxidase-enzyme conjugate will be added, which will bind highly specific to the biotin of the antiserum and will catalyse in the closing substrate reaction the turn of the colour, quantitatively depending on the hGH level of the sample.

The Standards of ELISA E02 are prepared from recombinant human hGH in concentrations of **1, 5, 10, 15 to 25 ng/ml** (nano Gramm/ml equal to **3, 15, 30, 45 and 75 µIU/ml** see below).
The 2. International Standard for hGH, **NIBSC Code 98/574** was used as standard material (6). 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 standard material is recommended in line with the current standardisation efforts for hGH Immunoassays (7,8) !

The **recovery** of the recombinant hGH yielded in a buffer matrix 100%. The recovery in different human-sera and in human urine samples was between 94% and 110% of the hypothetical expected amount.

The **linearity** of the serum dilution is over a very wide range excellent in the hGH ELISA.

Table 1: The linearity of the sample dilution

(characteristic results of three different sera, values recalculated in ng/ml)

Dilution:	**Sample 1**	**Sample 2**	**Dilution:**	**Sample 3**
undiluted	14.1	11.0	1:30	845,3
1:2	16.5	12.0	1:60	778,5
1:4	16.2	12.2	1:120	732,4
1:8	16.0	12.2	1:240	664,1
1:16	15.1	13.9	1:480	738,0
1:32	14.5	12.8	1:960	733,4
1:64	14.9	12.8		
AV / 1SD	15.3 / 0.92	12.4 / 0.88	**AV / 1SD**	748,6/ 60,0
VC%	5,98	7,07	**VC%**	8,01

AV = Average Value , SD = Standard deviation, VC = Variation coefficient%

The **analytical sensitivity** of the ELISA RMEE02R yields **0,25 ng/ml** (equal to 250 pg/ml, equal to 30 pg per well; 2x SD of zero standards in 16fold determination).

The **Inter**- and **Intra-Assay** Variation coefficients were found less than **7.0%** and **5.0%** respectively. Exemplary determinations are shown in table 2 and table 3.

Table 2: Inter-Assay-Variation

	Number of independent determinations	**Mean value (ng/ml)**	**Standard deviation (ng/ml)**	**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**

Table 3: Intra-Assay-Variation

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

Measurement of hGH during simultaneous application of hGH and hGH-analoga e.g. Pegvisomant (e.g. Somavert® of the Pfizer Inc.) is with the RMEE02R ELISA possible. The cross reaction of the assay was found to be less than 0.25% in serum levels of Pegvisomant (Pfizer; Somavert Insert Oct. 2003) detected in the long-term studies.

Samples: Applicability, Preparation and Storage

Serum as well as plasma samples are suitable (significant deviation of hGH levels in corresponding Serum, Heparin-, EDTA- or Citrate-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: as fast as possible and chilled as soon as possible. In case there will be a longer period between the sample withdrawal and determination store the undiluted samples frozen at –20°C or below in tightly closable plastic tubes. Avoid on principal repeated freeze-thaw cycles of serum/plasma (if required, please subaliquote) although hGH levels were found to be unaffected by few cycles (5x) in our experiments.

In most determinations (serum or plasma samples, and no extreme values expected) the sample size of 20 µl, undiluted, is optimally suitable. The hGH concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatants.

If necessary samples can be diluted in **Dilution Buffer VP**. For the determination of samples with very low hGH-levels the hGH-Sensitiv ELISA RMEE022R might be better suited (Sensitivity 0.0016 ng/ml ≈ 1.6 pg/ml).

MATERIALS

Materials Provided

1) **Microtiter plate**, ready for use: Microtiter plate with **96 wells**, divided up in 12 strips with 8 wells (**separately breakable)** Coated with an antibody against human Growth Hormone.

2) **Standards A-E**, 250 µl, ready for use: Contain recombinant hGH (NIBSC 98/574). Standard values are between **1 ng/ml – 25 ng/ml** (1,5,10,15 and 25 ng/ml) hGH 20 µl, per well are used in the assay.

3) **Dilution Buffer VP**, 30 ml, ready for use, please use for the **sample** dilution, if necessary.

4) **Control Serum KS**, 250 µl, ready for use: Contains human serum. The hGH target value concentration and the respective range are given on the vial label. The dilution of the KS should be according to the dilution of the respected samples, generally undiluted 20 µl/well.

5) **Antibody Conjugate AK,** 12 ml, ready for use solution, contains biotinylated anti-hGH antibody.

Use 100 µl of this solution per well in the assay.

6) **Enzyme Conjugate EK,** 12 ml, ready for use solution, contains HRP (Horseradish-Peroxidase)-conjugated Streptavidin.

Use 100 µl of this solution per well in the assay.

7) **Washing Buffer WP**, 50 ml, 20-fach concentrated solution; Washing Buffer has to be diluted **1:20** with distilled or demineralised water before use (e.g. add the complete contents of the flask (50 ml) into a graduated flask and fill with A.dest. to 1000 ml). Attention: After dilution the **Washing Buffer** is only limited stable, please dilute only according to requirements.

8) **Substrate S,** 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2-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.

TECHNICAL RECOMMENDATIONS

In conducting the assay, follow strictly the test protocol.

Reagents with different lot numbers should not be mixed.

The microtiter plate and all reagents are stable until the expiry date if stored in the dark at 2-8°C (s. label).

The **shelf life** of the components **after opening** is not affected, if used appropriately. Store the unused seal stripes of the microtiter plate together with the desiccant at 2-8°C.

The 1:20 diluted **Washing Buffer WP** is stable only limited. Please dilute only according to requirements.

Before use, all kit components should be brought **to room temperature**. Room temperature incubation means: incubation at 20 - 25°C. Precipitates in buffers should in case be dissolved before use thorough mixing and warming.

Incubation at room temperature means: Incubation at 20-25°.

The **Substrate Solution S**, stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

When performing the assay, the **Standards** (**A-E**), **Control Serum** (**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 the **Substrate Solution** (**S**)

Materials not provided

Distilled or demineralised water for dilution of the **Washing Buffer** (**WP**)

Micropipettes and multichannel pipettes with disposable plastic tips

Vortex-mixer

Device to aspirate the standards and the samples from the wells (recommended because of the potential danger of infection by human samples)

Plate washer and plate shaker (recommended)

Micro plate reader ("ELISA-Reader") with filter for 450/620nm (or ≥590 nm)

Foil welding device for laminate bags (recommended)

PRECAUTIONS

General

All reagents are for in vitro use only!

The acquisition, possession and use of the kit are subjects to the regulations of the national regulatory authorities.

First aid procedures:

Skin contact: Wash affected area thoroughly with water. Discard contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.

Ingestion: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.

The handling of potentially infectious human material (in the test kit only the provided **Control Serum KS**, has been shown negative for HBsAg, anti-HIV-1 and –2 and the individual samples) must comply

with the following guidelines:

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

Reagents contain as preservatives

ProClin 950

Following components contain ProCline 950 : **A-E, AK, VP**

< 0,1% 2-Methyl-4-isothiazolin-3-one Solution

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

Kathon CG

Following components contain Kathon CG: **A-E, AK, VP, WP**

< 0,1% (w/w) 5-chloro-2-methyl 2H isothiazol-3-one und 2-methyl-2H-isothiazol-3-one

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

Stop solution contains 0.2 M Sulfuric Acid (H_2SO_4)

R36/38	Irritating to eyes and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves.

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine. Store and Incubate in tge dark.

R20/21/R22	Harmful by inhalation, in contact with skin and if swallowed
R36/37/38	Irritating to eyes, respiratory system and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water

S36/37 Wear suitable protective clothing and gloves

ASSAY PROCEDURE

All determinations (**Standards**, **Control Serum** 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 VP.

1) Add additional **20µl Dilution Buffer VP** in wells A1/A2 (blank).

2) Pipette in positions B1/2 **20µl of Standard A (1 ng/ml)**

   Pipette in positions C1/2 **20 µl Standard B (5 ng/ml)**,

   Pipette in positions D1/2 **20 µl Standard C (10 ng/ml)**,

   Pipette in positions E1/2 **20 µl Standard D (15 ng/ml)**,

   Pipette in positions F1/2 **20 µl Standard E (25 ng/ml)**.

   To control the correct accomplishment **20 µl** of the undiluted (or in respective dilution rate of the sample) **Control Serum KS** can be pipetted in positions G1/2.

   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 **250 µl Washing Buffer WP** / well.

5) Following the last washing step, pipette **100 µl** of the **Antibody Conjugate AK** in each well and **incubate 30 minutes** (if possible, shake at ≥ 350 rpm)

6) Subsequently –**without a washing step!** - pipette **100 µl** of the **Enzyme-Conjugate** in each well 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** (**WP**) as described in step 4.

8) Pipette **100 µl** of the **TMB Substrate Solution 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 **Stopping Solution** (**SL**)

10) Measure the colour reaction within 30 minutes at 450 nm (reference filter 620 nm).

EVALUATION OF RESULTS

Establishing the Standard Curve

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.25, these of standard E should exceed 1,0. Samples, which yield higher absorbance values as **Standard E**, are beyond the standard curve, for reliable determinations these samples should be tested anew with a higher dilution.
The standards provided contain the following concentration of hGH:
(2. Internationaler Standard, NIBSC Code 98/574, 3 IU/mg):

Standard	**A**	**B**	**C**	**D**	**E**
ng/ml	1	5	10	15	25
µIU/ml	3	15	30	45	75

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 other values.
3) Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
4) Recommendation: Calculation of standard curve should be done by using a computer programme, because the curve is in general (without respective transformation) not ideally described by linear regression. Non-linear-Regression, a higher-grade polynomial or four parametric logistic (4PL) lin-log curve fit are suitable for the evaluation.
5) The hGH concentration in ng/ml (or µIU/ml, according the choosen unit for the standards) of the samples can be calculated by multiplication with the respective dilution factor.

Interpretation of results

The cut-off value is determined as a maximal peak of growth hormone secretion in at least 2 independent stimulation assays (e.g. insulin or arginine stimulation). Using WHO standard 98/574, which is equivalent to

standard material used in this assay, a secretion peak of less than **8 ng/ml** indicates a possible growth hormone deficiency.

But as growth hormone secretion is continous between normal and pathological any cut-off is only a non-binding benchmark.

Further diagnostic measurements should be carried out to approve the results of this test. And every laboratory should establish its own cut-off values corresponding to the relevant group of patients.

EXPECTED NORMAL VALUES

As growth hormone is secreted pulsatile mainly enduring the night sleep valid normal values can hardly be determined. Standard procedures are arginin 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.

	female	**male**
number	54	50
median [ng/ml]	0,81	0,28
minimal concentration [ng/ml]	0,19	0,15
maximal concentration [ng/ml]	10,15	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

SUMMARY: hGH ELISA (RMEE02R)

Reagents preparations	
The ready for use reagents: **Standard A – E** (each 250 µl), **Control serum KS** (250 µl), **Antibody conjugate AK** (12ml), **Enzyme conjugate EK** (12ml)	Bring to room temperature
Washing buffer (50 ml)	**1:20 with Aqua. dest.** (e.g., add the complete contents of the flask **(50 ml** into a graduated flask and fill with A.dest. to 1000 ml).
Samples	**Dilution is generally not necessary,** just use **20 µl** per single determination.

Proposal of Assay Procedure for double determinations:

Pipette	Reagents	Position
100 µl	Dilution Buffer **VP**	Pipette in all **required number of wells**
20 µl	Dilution buffer **VP** as blank	A1 and A2
20 µl	Standard **A** (**1 ng/ml**)	B1 and B2
20 µl	Standard **B** (**5 ng/ml**)	C1 and C2
20µl	Standard **C** (**10 ng/ml**)	D1 and D2
20 µl	Standard **D** (**15 ng/ml**)	E1 and E2
20 µl	Standard **E** (**25 ng/ml**)	F1 and F2
20 µl	Kontrollserum **KS**	G1 and G2
20 µl	Sample	Pipette sample in the rest of the wells according the requirements (**Pipetting Control** = colour turns red!)
Cover the wells with the sealing tape.		
Incubation: 1 h at RT, ≥ 350 rpm		
3x 250 µl	Aspirate the contents of the wells and wash 3x with **250 µl** Wash Buffer **WP**	in each well
100 µl	Antibody conjugate **AK**	in each well
Incubation: 30 min at RT, ≥350 rpm		
100 µl	Enzyme conjugate **EK, without washing the wells (!)** – add to the previously pipetted AK-solution **thereto,** thereby simultaneously mixing or mix shortly through cautious tapping on the MTP. **Attention: high filled volume of the wells!**	in each well
Incubation: 30 min at RT, without shaking.		
3x 250 µl	Aspirate the contents of the wells and wash 3x with **250 µl** Wash Buffer **WP**	in each well
100 µl	Substrate solution **S**	in each well
Incubation: 15 min in the dark at RT		
100 µl	Stopping solution **SL**	in each well
Measure the absorbance within **30 min** at **450 nm** with **≥590 nm** as reference wavelength.		

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