Elecsys hGH

cobas®

REF		Σ	SYSTEM
07027486190	07027486500	100	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
HGH	10096

Intended use

Immunoassay for the in vitro quantitative determination of human growth hormone (hGH; forms with molecular masses of 20 kDa and 22 kDa) in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

HGH measurements (forms with molecular masses of 20 kDa and 22 kDa), performed with this assay, in human serum and plasma, are used as an aid in the assessment of growth disorders in conjunction with other clinical and laboratory findings.

HGH is a hormone that is produced by the somatotroph cells of the pituitary gland and its major physiological effect is to promote growth in soft tissue, cartilage and bone.[1] HGH (directly) or together with its main mediator (indirectly), Insulin-like Growth Factor 1 (IGF-1), modulates bone and muscle growth and differentiation.[1,2] HGH has also other general anabolic effects such as increase of glucose uptake, protein synthesis, lipolysis. The balance between the Growth Hormone-releasing Hormone (GHRH) and the somatotropin release-inhibiting hormone (SRIH), secreted by the hypothalamus, regulates the release of hGH.[3,4] Leptin from adipose tissue and ghrelin from the stomach can directly increase the concentration of hGH by stimulating the hypothalamic release of GHRH.[5] Other hGH stimulators are sleep, physical exercise, insulin, low levels of blood sugar, increased androgen secretion during puberty. The release of hGH is also inhibited by glucose, glucocorticoids, fatty acids, L-dopa and beta-blockers and is further regulated by circulating concentrations of hGH and IGF-1 by a negative feedback mechanism. HGH secretion is further under the influence of additional hormonal signals, sex steroids and thyroid hormone stimulation.[2,6]

HGH is a heterogeneous protein composed of several molecular isoforms. The isoform with a molecular mass of 22 kDa is the most abundant, representing more than 90 % of the circulating hGH. The 20 kDa isoform, lacking the 32-46 amino acid residues, is the second most abundant hGH isoform, representing about 10 % of the total circulating hGH.[7]

Growth hormone excess is typically associated with gigantism and acromegaly. Gigantism is an abnormal high linear growth due to excessive action of hGH and IGF-1 while the epiphyseal growth plates are open during childhood resulting in tall stature.[8] Acromegaly is the same disorder of hGH and IGF-1 excess when it occurs after the growth plate cartilage fuses in adulthood. It is frequently caused by hGH secreting somatotrope adenomas of the anterior pituitary gland.[9] The clinical manifestations of acromegaly range from subtle signs, such as acral overgrowth and coarsening of facial features, to significant metabolic, cardiovascular, and respiratory manifestations, leading to an increase in morbidity and mortality.[10,11]

Growth hormone deficiency (GHD) in children results in retardation of longitudinal growth compared to bone age whereas severe GHD in adults is associated with reduced muscle strength and bone mass, insulin sensitivity, abdominal adiposity and increased cardiovascular risk factors (i.e. abnormal lipid profile, atherosclerosis).[1,12,13,14,15,16] With progressing, GHD adults show renal, skeletal and intestinal cell insensitivity to parathyroid hormone (PTH) leading to a mild state of PTH resistance and increased serum PTH levels.[17] Consistent with a decrease in end-organ sensitivity, the calcemic response to PTH is delayed.[18]

Clinical interpretation of growth hormone levels must be done with caution, as hGH levels vary throughout the day, between genders, are age-related and are influenced by many internal and external factors (exercise, stress, hypoglycemia, etc.). The diagnosis of hGH deficiency or excess is based on clinical-auxological criteria and MRI imaging of the pituitary gland. It is confirmed by a determination of hGH concentration in serum via stimulation or suppression tests (i.e. a combination of arginine and GHRH, clonidine or insulin).[19,20,21,22] Cutoff levels for the diagnosis of hGH deficiency vary depending on the type of stimulation test and are influenced by the body mass index (BMI).[23] Guidance on cutoff levels should be taken from literature.[19,20,21,22,23]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 24 µL of sample, a biotinylated monoclonal hGH-specific antibody and a polyclonal hGH-specific antibody labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as HGH.

M Streptavidin-coated microparticles, 1 bottle, 6.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-hGH-Ab~biotin, 1 bottle, 7.6 mL:
Biotinylated monoclonal anti-hGH antibody (mouse) 1.1 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-hGH-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 7.2 mL:
Polyclonal anti-hGH antibody (sheep) labeled with ruthenium complex 2.4 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

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P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept ≤ ± 0.04 ng/mL + coefficient of correlation ≥ 0.9.

Stable for 8 hours at 20-25 °C, 1 day at 2-8 °C, 1 month at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 05390133190, hGH CalSet, for 4 x 1.0 mL
- REF 05341787190, PreciControl Multimarker, for 6 x 2.0 mL or REF 07476108190, PreciControl Growth, for 4 x 3.0 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the IRP (International Reference Preparation), NIBSC (National Institute for Biological Standards and Control) code 98/574.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

Use PreciControl Multimarker or PreciControl Growth or other suitable controls for routine quality control procedures.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL, pg/mL or mIU/L).

Conversion factors:	ng/mL x 1000 = pg/mL
	pg/mL x 0.001 = ng/mL
	ng/mL x 3.0 = mIU/L
	mIU/L x 0.333 = ng/mL

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 428 µmol/L or ≤ 25 mg/dL
Hemoglobin	≤ 0.310 mmol/L or ≤ 500 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 123 nmol/L or ≤ 30 ng/mL
Rheumatoid factors	≤ 600 IU/mL
IgG	≤ 3.5 g/dL
IgA	≤ 0.85 g/dL
IgM	≤ 0.55 g/dL

Criterion: For concentrations of 0.030-0.7 ng/mL the deviation is ≤ 0.08 ng/mL. For concentrations > 0.7 ng/mL the deviation is ≤ 12 %.

Do not use samples that show visible signs of hemolysis.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

There is no high-dose hook effect at hGH concentrations up to 2000 ng/mL.

Pharmaceutical substances
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found. No interference was found for L-Thyroxin.

The assay is affected by pegvisomant (a highly selective GH receptor antagonist) and is therefore not suitable for patients under pegvisomant treatment. There is no interference with Octreotide (somatostatin analogue) or Cabergoline (dopamine agonist).

The assay is not suitable for the determination of hGH in samples from pregnant women due to a cross-reactivity to placental hGH. Placental hGH is a variant of pituitary hGH[24] and its serum levels increase during pregnancy.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.030-50.0 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.030 ng/mL. Values above the measuring range are reported as > 50.0 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.020 ng/mL

Limit of Detection = 0.030 ng/mL

Limit of Quantitation = 0.050 ng/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples with hGH concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (either automatically by the analyzer or manually). The concentration of the diluted sample must be > 25 ng/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzer, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

Basal levels of hGH do not have a diagnostic relevance and stimulation tests are needed (see above) to assess a growth hormone disorder. Therefore the following values from healthy subjects are for information only and should not be used for diagnostic purposes.

Percentiles	Girls (n = 43) 0-10 years, median: 5 years	Boys (n = 86) 0-10 years, median: 5 years
	hGH (ng/mL)	
5	0.120	0.094
50	0.689	0.814
95	7.79	6.29

Percentiles	Girls (n = 38) 11-17 years, median: 15 years	Boys (n = 33) 11-17 years, median: 13 years
	hGH (ng/mL)	
5	0.123	0.077
50	0.432	0.322
95	8.05	10.8

Percentiles	Women (n = 150) 21-77 years, median: 50 years	Men (n = 149) 20-79 years, median: 50 years
	hGH (ng/mL)	
5	0.126	< 0.030
50	0.944	0.119
95	9.88	2.47

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.043	0.002	5.0	0.003	5.9
Human serum 2	0.054	0.002	4.1	0.002	4.3
Human serum 3	8.12	0.224	2.8	0.242	3.0
Human serum 4	25.0	0.321	1.3	0.421	1.7
Human serum 5	41.8	0.933	2.2	1.05	2.5
PC[b)] Multimarker 1	0.892	0.007	0.8	0.009	1.1
PC Multimarker 2	8.83	0.050	0.6	0.099	1.1

b) PC = PreciControl

Method comparison

a) A comparison of the Elecsys hGH assay, REF 07027486190 (**cobas e** 801 analyzer; y), with the Elecsys hGH assay, REF 05390125190 (**cobas e** 601 analyzer; x), gave the following correlations (ng/mL):

Number of samples measured: 166

Passing/Bablok[25]	Linear regression
y = 0.993x + 0.0048	y = 1.01x - 0.044
τ = 0.982	r = 1.000

The sample concentrations were between 0.030 and 48.3 ng/mL.

b) A comparison of the Elecsys hGH assay, REF 07027486190 (**cobas e** 402 analyzer; y), with the Elecsys hGH assay, REF 07027486190 (**cobas e** 801 analyzer; x), gave the following correlations (ng/mL):

Number of samples measured: 146

Passing/Bablok[25]	Linear regression
y = 1.00x + 0.002	y = 0.995x + 0.008
τ = 0.990	r = 1.000

The sample concentrations were between 0.048 and 47.9 ng/mL.

Analytical specificity

The following cross-reactivities were found, tested at hGH concentrations of 1 ng/mL and 10 ng/mL:

	Concentration tested	Cross-reactivity %
TSH	100 µIU/mL	≤ 0.672
FSH	200 µIU/mL	≤ 1.30
LH	200 mIU/mL	≤ 1.32
hCG	10000 mIU/mL	≤ 0.025
Prolactin	470 ng/mL	≤ 0.544
hPL	40 ng/mL	≤ 0.730
IGF-1	900 ng/mL	≤ 0.161
hGH isoform 20 kDa (WHO: 80-505)	100 ng/mL	≥ 75.4

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For further information, please refer to the appropriate user guide or operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Elecsys hGH

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Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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