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DCM050-8
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HGH ELISA

for routine analysis

Quantitative determination of Human Growth Hormone (HGH) in human serum or plasma

IVD

LOT
See external label

2°C 8°C

Σ = 96 tests REF DKO050

INTENDED USE

Eagle Biosciences HGH ELISA Assay kit is a direct solid phase enzyme immunoassay for the quantitative measurement of Growth Hormone in human serum or plasma. HGH ELISA Assay kit is intended for research use only.

1. CLINICAL SIGNIFICANCE

Growth hormone is a polypeptide hormone synthesised and secreted by the anterior pituitary gland which stimulates growth and cell reproduction in humans and other vertebrate animals. GH is released from the pituitary into the bloodstream in a pulsatile manner under the regulatory control of hypothalamic somatostatin (SS) and GH-releasing factor (GHRF). Stimulators of GH secretion include, exercise, hypoglycemia, dietary protein, and estradiol. Inhibitors of GH secretion include dietary carbhoydrate and glucocorticoids.

Almost 50% of GH in circulation is bound to a high affinity GH binding protein (GHBP), which represents the extracellular domain of the GH receptor. The plasma concentration of GH during these peaks may range from 5 to 30 ng/mL or more. Peaks typically last from 10 to 30 minutes before returning to basal levels. The largest and most predictable of these GH peaks occurs about an hour after onset of sleep. Otherwise there is wide variation between days and individuals. Between the peaks, basal GH levels are low, usually less than 3 ng/mL for most of the day and night.

The amount and pattern of GH secretion change throughout life. Basal levels are highest in early childhood. The amplitude and frequency of peaks is greatest during the pubertal growth. Healthy children and adolescents average about 8 peaks per 24 hours. Adults average about 5 peaks. Basal levels and the frequency and amplitude of peaks decline throughout adult life.

The primary biological actions of the hormone are in direct growth promoting. HGH exerts its effect directly on target organs such as cartilage, bones and muscles and indirectly through the release of insulin-like growth factor (IGF), produced in the liver (2). In particular, somatotropin C (IGF-1) is essential for bone growth during childhood.

Generally increases protein synthesis and stimulates the growth of all internal organs excluding the brain. GH reduces liver uptake of glucose, an effect that opposes that of insulin. GH also contributes to the maintenance and function of pancreatic islets. GH stimulates the immune system. GH can cause excessive growth, traditionally referred to as pituitary giganttism.

Deficiency of GH produces significantly different problems at various ages. In children, growth failure and short stature are the major manifestations of GH deficiency. In adults the effects of deficiency are more subtle, and may include deficiencies of strength, energy, and bone mass, as well as increased cardiovascular risk.

2. PRINCIPLE OF THE METHOD

In this HGH ELISA Assay Kit, the calibrators and the patient specimens and/or controls (containing the native HGH antigen) are first added to streptavidin coated wells. Biotinylated monoclonal and enzyme labelled antibodies are then added: these antibodies have high affinity and specificity and are directed against distinct and different epitopes of HGH.
Reaction between the various HGH antibodies and native HGH occurs in the microwells without competition or steric hindrance, forming a soluble sandwich complex.
The interaction is illustrated by the following equation:

$$EAb(x\text{-}GH) + AgGH + BtAb(m) \underset{k\text{-}a}{\overset{ka}{\leftrightarrow}} EAb(x\text{-}GH)\text{-}AgGH\text{-}BtAb(m)$$

BtAb(m) = Biotinylated Monoclonal Antibody (Excess Quantity)
AgGH = Native Antigen (Variable Quantity)
EAb(p) = Enzyme labelled Antibody (Excess Quantity)
EAb(x-GH)-AgGH-BtAb(m)= Sandwich Complex
ka = Rate Constant of Association
k-a = Rate Constant of Dissociation

Simultaneously, the complex is immobilized in the well through the high affinity reaction of streptavidin and biotinylated antibody.
This interaction is illustrated below:

EAb(x-GH)-AgGH-BtnAb(m)+Strept.C.W.→

Immobilized complex

Strept.C.W. = Streptavidin immobilized on the well
Immobilized complex = sandwich complex bound to the well.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. Then the activity of the enzyme HRP present on the surface of the well is quantified by reaction with the TMB Substrate to produce color. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve is generated from which the antigen concentration of an unknown is ascertained.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. HGH Calibrators (6 vials, 1 mL each)

CAL0	**REF DCE002/5006-0**
CAL1	**REF DCE002/5007-0**
CAL2	**REF DCE002/5008-0**
CAL3	**REF DCE002/5009-0**
CAL4	**REF DCE002/5010-0**
CAL5	**REF DCE002/5011-0**

2. HGH Control (1 vial, 1 mL)
Concentration of Control is Lot-specific and is stated on Quality Control Report **REF DCE045/5003-0**

3. Conjugate (1 vial, 13 mL)
Antibodies anti HGH conjugated with horseradish peroxidase (HRP) and anti HGH biotinylated
REF DCE002/5002-0

4. Coated Microplate (1 breakable microplate)
Microplate coated with streptavidin
REF DCE002/5003-0

5. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
REF DCE004-0

6. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15 mol/L *(avoid any skin contact)*
REF DCE005-0

7. 50X Conc. Wash Solution (1 vial, 20 mL)
NaCl 45 g/L; Tween-20 55 g/L **REF DCE006-0**

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm)

Note

Store all reagents between 2-8°C in the dark.

Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until the expiry date of the kit.

4. WARNINGS

- This HGH ELISA Assay Kit is intended for research use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents of the HGH ELISA Assay Kit contain small amounts of Proclin 300^R as preservatives. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This HGH ELISA Assay Kit allows the determination of HGH from 2 μIU/mL to 150 μIU/mL.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents of the HGH ELISA Assay Kit should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all HGH ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange HGH ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate

- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C_0…C_5)

The Calibrators are ready for use and have the following concentration:

	C_0	C_1	C_2	C_3	C_4	C_5
µIU/mL	0	2	10	25	50	150

For sample with concentration over 150 µIU/mL dilute the sample with the Calibrator 0.
Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of Wash Solution

Dilute the content of each vial of the "50X Conc. Wash Solution" with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

6.3. Preparation of the Sample

The specimens shall be human plasma or serum; the usual precautions in the collection of venipuncture samples should be observed.
For accurate comparison to establish normal values, a fasting morning serum sample should be obtained.
For serum preparation, the blood should be collected in a venipuncture tube without additives or anti-coagulants; allow the blood to clot; centrifuge the specimen to separate the serum from the cells.
Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimens cannot be assayed within this time, they may be stored -20°C for up to 30 days. Avoid repetitive freezing and thawing.
When assayed in duplicate, 0,100 mL of the specimen is required.
The Control is ready to use.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C)**
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Control	Blank
Calibrator C_0-C_5	**50 µL**		
Sample/ Control		**50 µL**	
Conjugate	**100 µL**	**100 µL**	
Incubate at room temperature (22-28°C) for 1 hour. Remove the content from each well and wash the wells 3 times with 300 µL of Diluted Wash Solution.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22-28°C) for 15 minutes in the dark .			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank.			

7. QUALITY CONTROL

Each laboratory should assay controls at levels of a low, normal, and high range for monitoring assay performance.
These controls should be treated as unknowns and values determined in every test procedure performed.
Quality control charts should be maintained to follow the performance of the supplied reagents.
Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents.
Fresh reagents should be used to determine the reason for the variations.

8. LIMITATIONS OF PROCEDURE

8.1. Assay Performance

The administration of HGH to patients may develop antibodies anti-HGH that may interfere with the test and give false low results. Genetic variants or degradation products may alter the binding characteristics of the antibodies and influence the final results. These samples may show discordant results when tested with different methods, based on antibodies binding to different epitopes.
The HGH secretion follows a circadian rhythm that is characterized by a discontinuous and pulsatile release of the hormone, with HGH concentration increase alternated to periods during the day in which

the HGH concentration is undetectable. The highest concentrations, in two major peaks of secretion, are usually reached within one or two hours after the onset of sleep. Other physiological HGH stimuli are stress, exercise, meals with high protein content and hypoglycaemia.
Hyperglycemia inhibits the secretion of growth hormone. Age is an important factor influencing its concentration. At birth, the HGH is high, and generally it decreases with age, with the exception of a peak in the growth phase of adolescence. Women usually have a 50% concentration than their male peers.
As the concentration of HGH is pulsatile and intermittent during the day and the hormone has a very short half-life, the levels of HGH determined on individual random tests do not provide information of clinical utility. To work around this problem, stimulation tests using pharmacological and physiological stimuli to induce the secretion or inhibition of HGH are used. For these reasons, the determination of growth hormone alone is not sufficient to establish the clinical conditions.

9. RESULTS

9.1. Note

Optical densities (O.D.) higher than 2.0 could be out of the measurement range of some microplate readers. It is therefore necessary, for O.D.s higher than 2.0, to perform a reading at 405 nm (=wavelength of peak shoulder) in addition to 450 nm (peak wavelength) and 620 (reference filter for the subtraction of interferences due to the plastic).
For microplate readers unable to read the plate at 3 wavelengths at the same time,
it is advisable to proceed as follows:
- Read the microplate at 450 nm and at 620 nm.
- Read again the plate at 405 nm and 620 nm.
- Find out the wells whose ODs at 450 nm are higher than 2.0
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where OD 450/OD 405 = 3.0), that is: *OD 450 nm = OD 405 nm x 3.0*

Warning: The conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for his reader.

9.2. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (C_0-C_5) and of each sample.

9.3. Calibration curve – Automatic method

Use the 4 parameters logistic – preferred – or the smoothed cubic spline function as calculation algorithm.

9.4. Calibration curve – Manual method

A dose response curve is used to ascertain the concentration of growth hormone levels in unknown specimens.
Record the optical density (OD) obtained from the printout of the microplate reader.
Plot the OD for each duplicate calibrator versus the corresponding HGH concentration in µIU/mL on linear graph paper (do not average the duplicates of the serum references before plotting).
Draw the best-fit curve through the plotted points.
To determine the concentration of HGH for an unknown, locate the average OD of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µIU/mL) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

10. REFERENCE VALUES

Because of the pulsatile and sporadic nature of growth hormone secretion, reference intervals for basal values are without meaning. However, normal levels rarely have been reported above 150 µIU/mL.
Keeping in mind this premis, 175 apparently healthy adults were assayed the HGH immunoassay. The results are depicted in Table:

	N.	Mean µIU/mL	Range µIU/mL
Samples	175	9.1	0-55

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

Provocative tests for HGH response are normally used to access the function of the anterior pituitary. Stimulatory procedures measure the secretion ability of the anterior pituitary to release HGH. Children suspected of growth retardation are common subjects for stimulatory testing. Several dynamic tests are available to induce HGH release: exercise, L-dopa administration, insulin tolerance test, and arginine infusion. Each laboratory should assess the normal response, but a peak HGH release in excess of 24µIU/mL is probably normal in all cases.
Inhibitory testing measure the suppression of HGH release from the anterior pituitary. Inhibitory tests are useful in ascertaining growth hormone excess and the resulting conditions of gigantism and acromegaly.
The glucose tolerance test is a dynamic test to measure growth hormone excess. The failure of HGH levels to fall below 1µIU/mL within 60-120 minutes suggests excess HGH secretion.

11. PERFORMANCE AND CHARACTERISTICS

11.1. Precision

11.1.1. Intra Assay Variation

Within run variation was determined by replicate the measurements (24x) of three different control sera in one assay.

Sample	N.	X	σ	CV
Level 1	24	10.38	0.33	3.13%
Level 2	24	26.23	1.17	4.45%
Level 3	24	61.80	3.40	5.50%

11.1.2. Inter Assay Variation

Between run variations was determined by replicate (39x) the measurements of three different control sera in different lots of the kit.

Sample	N.	X	σ	CV
Level1	39	10.48	0.48	4.58%
Level2	39	26.08	1.77	6.79%
Level3	39	64.61	4.58	7.09%

11.2. Sensitivity

The lowest detectable concentration of HGH that can be distinguished from the Calibrator 0 is 0.105 μIU/mL at the 95% confidence limit.

11.3. Specificity

In order to assess the specificity of the antibody pair used for the HGH Elisa assay, massive doses of related analytes were spiked in a pool of patient sera:

Growth Hormone (HGH)	1.0000
Luteinizing Hormone (LH)	< 0.0001
Follicle Stimulating Hormone (FSH)	< 0.0001
Thyroid Stimulating Hormone (TSH)	< 0.0001
Prolactin Hormone (PRL)	< 0.0001
Chorionic gonadotropin (CG)	< 0.0001

11.4. Correlation with RIA method

The Diametra HGH ELISA was compared to another commercially available HGH assay. Serum samples of 80 subjects were analysed according in both test systems. The linear regression curve was calculated:

(HGH Diametra) = 0.091 + 0.98*(HGH RIA)
Correlation coefficient= 0.985

12. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation