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Growth Hormone ELISA Kit

Name	Growth Hormone ELISA Kit
INTENDED USE	The Calbiotech, Inc. (CBI) hGH ELISA kit is used for the quantitative measurement of hGH in human serum or plasma.
SUMMARY AND EXPLANATION	Human Growth Hormone (hGH) is a polypeptide chain, composed of 191 amino acids and with a molecular weight of 21,500. It is released by the anterior pituitary of both men and women. The secretion is stimulated 3-4 hours after a meal, about 1 hour after the beginning of sleep and after physical exercise. Hyposecretion of hGH becomes apparent in infants a few months after birth and may result in dwarfism. In the opposite case, hypersecretion of hGH results in gigantism and may be due to hypophysic tumors. In adults, when epiphyses are closed, hypersecretion of hGH provokes an increase in volume of soft tissues (hands, feet, lips) and a proliferation of bones (acromegalysyndrome) and a limited tolerance of glucose. hGH has profound effects on tissue growth and metabolism, which is thought to be mediated through GH-dependent production of Insulin-like Growth Factor (IGF) I and IGF-II, and their associated binding proteins. hGH apparently stimulates IGF production after binding to specific cell surface receptors in the liver. The major target tissues affected by the IGF-1 in combination with the hGH signal are muscle, cartilage, bone, liver, kidney, nerves, skin and lungs. Evaluation of hGH deficiency is complicated by the episodic nature of hGH secretion and low circulating levels. A variety of physiologic and pharmacologic stimuli have been used to stimulate pituitary hGH release during testing and failure to achieve a normal serum hGH level in response to at least 2 hGH

stimulation or provocative tests is considered to be a diagnostic of hGH deficiency. The definition of a normal serum hGH response is controversial, although published values generally range from 5 to 10 ng/ml.

PRINCIPLE OF THE TEST

The Calbiotech Inc., hGH ELISA is based on solid phase sandwich hGH method. The samples and conjugate reagent (anti-hGH biotin & HRP) are added to the wells coated with Streptavidin. hGH in the patient's serum binds to the matched pair Abs, forming a sandwich complex and simultaneously the complex is being immobilized on the plate through streptavidin-biotin interactions. Unbound protein and HRP conjugate are washed off, through a washing step. Upon addition of the substrate, the intensity of color is proportional to the concentration of hGH in the samples. A standard curve is prepared by relating the color intensity to the concentration of hGH.

MATERIALS PROVIDED	96 Tests
Microwells coated with Streptavidin	12x8x1
hGH Standard: 6 vials (ready to use)	0.5ml
hGH Conjugate Reagent: 1 bottle (ready to use)	12 ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
20X Wash concentrate: 1 bottle	25ml

MATERIALS NOT PROVIDED

Distilled or deionized water

precision pipettes

Disposable pipette tips

Micortiter well reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

Graph paper

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STORAGE AND STABILITY	1. Store the kit at 2 - 8 C. Keep microwells sealed in a dry bag with desiccant The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light.
WARNINGS AND PRECAUTIONS	Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. It is recommended that standards, control and serum samples be run in duplicate. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
SPECIMEN COLLECTION HANDLING	Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8 C) for 5 days. If storage time exceeds 5 days, store frozen at (-20 C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens.

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REAGENTS PREPARATION	Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25C).
ASSAY PROCEDURE	Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use. Place the desired number of coated strips into the holder Pipet 50 l of hGH standards, control and patient's sera onto appropriate wells. Add 100 l of hGH conjugate reagent to all wells. Cover the plate and incubate for 60 minutes at room temperature (20-25C). Remove liquid from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbent paper towels. Add 100 l of TMB substrate to all wells. Incubate for 15 minutes at room temperature. Add 50 l of stop solution to all wells. Shake the plate gently to mix the solution. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.
CALCULATION OF RESULTS	The standard curve is constructed as follows: Check hGH standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached. To construct the standard curve, plot the absorbance for the hGH standards (vertical axis) versus the hGH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample. Value above the highest point of the standard are retested after diluting with "0" standard.

Example of a standard Curve

Standard	OD (450 nm)
Standard 1 (0 µIU/ml)	0.006
Standard 2 (2 µIU/ml)	0.123

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Standard 3 (10 µIU/ml)	0.465
Standard 4 (25 µIU/ml)	1.004
Standard 5 (50 µIU/ml)	1.503
Standard 6 (150 µIU/ml)	2.328

LIMITATIONS OF THE TEST

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for hGH may be used as initial guideline ranges only:

Classification	Normal Range (ng/ml)
Adults	Less than 10 ng/ml
Children	Less than 20 ng/ml 7-10 ng/ml on two or more tests = impaired hGH secretion.

RESULTS

Results are expressed in µIU/mL. To convert to ng/mL, divide results by 3.7. Example:

10µIU/mL/3.7 = 2.7ng/mL

PERFORMANCE CHARACTERISTICS

Precision

Intra-Assay

Serum	No. of Replicates	Mean (µIU/mL)	Standard Deviation	Coefficient of Variation (%)
1	24	15.0	0.73	4.90
2	24	41.3	2.41	5.83
3	24	102	7.84	7.67

Inter-assay

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	24	14.3	0.65	4.53
2	24	33.7	1.69	5.01

3	24	100.4	8.63	8.59

Specificity

The antibodies used in this assay are highly specific for the detection of hGH. The following related analytes were screened for cross-reactivity.

Compound	Concentration	% Cross Reactivity
hGH	150µIU/mL	100%
hCG	125 IU/mL	ND
LH	500 mIU/mL	ND
Prolactin	500 ng/mL	ND
FSH	500 mIU/mL	ND
TSH	500 µIU/mL	ND

Sensitivity

This HGH test kit has a sensitivity of 0.036µIU/ml. Sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run assay and re-plotting it of off the dose response curve.

Recovery

hGH (µIU/mL)	hGH Spiked (µIU/mL)	Expected Value (µIU/mL)	Measured Value (µIU/mL)	Recovery (%)
38.6	74	112.6	113.1	100
38.6	37	75.6	81.9	108
38.6	18.5	57.1	56.1	98

Linearity

Two different patient samples were diluted with the “0” calibrator to 1:2, 1:4 and 1:8. hGH values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Original Value	Original Value	Percentage of Recovery	Percentage of Recovery	Percentage of Recovery
Serum	(µIU/ml)	1/2	1/4	1/8

1	62.13	107%	113%	117%
2	95.13	106%	110%	109%

REFERENCES

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