DRG® HGH (Human Growth Hormone) (EIA-1787)

CE

Revised 13 Sept. 2010 rm (Vers. 3.1)

IVD

Please use only the valid version of the package insert provided with the kit.

For In Vitro Diagnostic Use

Store at 2 to 8°C.

Intended Use

The HGH ELISA is intended for the quantitative determination of human growth hormone (HGH) concentration in human serum.

The test is useful in the diagnosis and treatment of disorders involving the anterior pituitary gland.

Explanation of the Test

Human Growth Hormone (hGH, somatotropin) is a polypeptide secreted by the anterior pituitary. It is 191 amino acids in length, has a molecular mass of approximately 22,000 daltons, and its metabolic effects are primary anabolic.[1] hGH promotes protein conservation and is engaged in a wide range of mechanisms for protein synthesis. It also enhances glucose transport and facilitates glycogen storage. Its cascade of growth-promoting action is mediated by another family of peptide hormones, the somatomedins.[2] hGH measurement is primarily of interest in the diagnosis and treatment of various forms of abnormal growth hormone secretion. Disorders caused by hyposecretion include dwarfism and unattained growth potential. Hypersecretion is associated with gigantism and acromegaly.[3]

Caution must be exercised in the clinical interpretation of growth hormone levels. These vary throughout the day, making it difficult to define a normal range or to judge an individual's status based on a single determination. Many factors are known to influence the rate of growth hormone secretion, including periods of sleep and wakefulness, exercise, stress, hypoglycemia, estrogens, corticosteroids and L-dopa. Because of its similarity to prolactin and placental lactogen, earlier GH immunoassays were often plagued with falsely high values in pregnant and lactating women.[2,3,6]

Growth hormone-deficient individuals have fasting and resting levels similar to those found in normal individuals. Various challenge tests have therefore been devised to differentiate them. For example, with the onset of deep sleep or after 15 to 20 minutes of vigorous exercise, growth hormone levels normally rise.[4]

Other tests of growth hormone responsiveness are based on the administration of L-dopa, arginine and insulin. Propanolol or estrogen are sometimes given in conjunction with the primary stimulus to accentuate the response.[4,5]

A small number of dwarfism cases have been documented in which both the basal level of HGH and the responses to challenge testing were normal. Such cases may involve tissue insensitivity to either growth hormone or the somatomedins, or immunoreactive but biologically inactive growth hormone.[4]

The Human Growth Hormone Enzyme Immunoassay provides a rapid, sensitive, and reliable test for GH measurement. There is no cross-reactivity with hCG, TSH, LH, FSH and prolactin

Principle of the Assay

The HGH ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a sheep anti-HGH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-HGH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in HGH molecules being sandwiched between the solid phase and enzyme-linked antibodies.

After a 45 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of HGH is directly proportional to the color intensity of the test sample.

Reagents and Materials Provided

1. **Antibody-Coated Wells** (1 plate, 96 wells)
   Microtiter wells coated with sheep anti-hGH.
2. **Enzyme Conjugate** Reagent (13 mL)
   Contains mouse monoclonal anti-hGH conjugated to horseradish peroxidase.
3. Reference **Standard** Set (1 mL/vial)
   Contains 0, 2.5, 5, 10, 25 and 50 ng/mL human growth hormone, ready to use.
4. **TMB Reagent** (1 bottle, 11 mL)
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.

5. **Stop Solution** (1N HCl) (1 bottle, 11 mL)
   Contains diluted hydrochloric acid.

Materials Required But Not Provided

- Distilled or deionized water
- Precision pipettes: 0.05, 1.0, 0.2, and 1 mL
- Disposable pipette tips
- Microtite well reader capable of reading abs. at 450 nm.
- Absorbent paper
- Graph paper
- Vortex mixer or equivalent
- Quality control material (e.g., BioRad Lyphochek Control sera)

Warnings and Precautions

1. **CAUTION:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[21]
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

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Storage Conditions

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

Instrumentation

A microtiter well reader with a bandwidth of 10nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

Specimen Collection and Preparation

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Aviod use of additives. Avoid grossly hemolytic, lipemic, or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C prior to assaying. Specimens held for a longer time (up to 6 months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Reagent Preparation

All reagents should be allowed to reach room temperature (18-25°C) before use, and should be mixed by gentle inversion or swirling. Do not induce foaming.

Procedural Notes

1. Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes. A multi-channel pipette is recommended.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

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Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Pipette 50 µL of standards, specimens, and controls into appropriate wells.
3. Add 100 µL of Enzyme Conjugate Reagent into each well.
4. Mix thoroughly for 30 seconds.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled H_2O.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µL of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature, in the dark, for 20 minutes.
11. Stop the reaction by adding 100 µL of Stop Solution to each well.
12. Gently mix for 30 seconds. ***Ensure that all of the blue color changes completely to yellow.***
13. Read absorbance at 450nm with a microtiter plate reader ***within 15 minutes***.

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Calculation of Results

1. Calculate the mean absorbance value (OD_{450}) from the duplicate set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of HGH in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

Standardization of Assay

The HGH Reference Standards are calibrated against the World Health Organization's First International Reference Preparation (WHO 1st IRP 66/217).

Example of Standard Curve

Results of a typical standard run with optical density readings at 450nm shown on the Y-axis against HGH (ng/mL) shown on the X-axis, are presented below. **NOTE:** the standard curve is for illustration only, and should not be used to calculate unknowns.

HGH (ng/mL)	Absorbance (450 nm)
0	0.052
2.5	0.392
5	0.641
10	1.125
25	1.946
50	2.610

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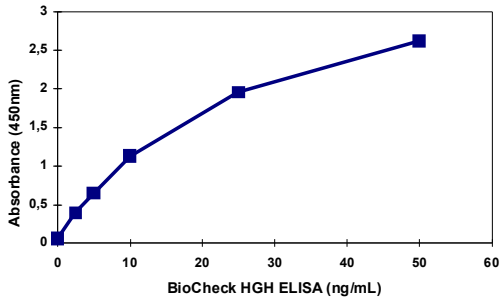

Expected Values

Each laboratory must establish its own normal ranges based on patient population.

A normal range for human growth hormone levels is difficult to define because of the normal physiological fluctuations in HGH concentration.[6] In most adult subjects at rest, after an overnight fast, the HGH level in serum is 7 ng/ml or less.

Changes in HGH levels in response to various stimuli gives a more accurate assessment of pituitary dysfunction.

QUALITY CONTROL

Good laboratory practice requires that low, medium and high quality control specimens (controls) be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges.

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Performance Characteristics

Accuracy

A statistical study using patient samples demonstrated good correlation of results with the commercially available kits as shown below:

a. Comparisons between DRG and DPC kits provide the following data:

N = 134
Correlation coefficient = 0.962
Slope = 1.041
Intercept = 0.115
DRG Mean = 1.780 ng/mL
DPC Mean = 1.970 ng/mL

b. Comparisons between DRG and Nichols Institute kits provide the following data:

N = 134
Correlation coefficient = 0.985
Slope = 0.860
Intercept = 0.119
DRG Mean = 1.780 ng/mL
Nichols Mean = 1.650 ng/mL

Sensitivity

The minimal detectable concentration of human growth hormone (HGH) by this assay is estimated to be 0.5 ng/mL.

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Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of three different control sera in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3
Number of Replicates	28	28	28
Mean HGH (ng/mL)	6.93	12.92	31.17
Standard Deviation	0.20	0.32	0.67
Coefficient of Variation (%)	2.9%	2.5%	2.2%

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of three different control sera in several different assays. Between-assay variability is shown below:

Serum Sample	1	2	3
Number of Replicates	28	28	28
Mean HGH (ng/mL)	6.55	11.90	30.85
Standard Deviation	0.21	0.52	0.91
Coefficient of Variation (%)	3.2%	4.4%	3.0%

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Recovery and Linearity Studies

a. Recovery

Various patient serum samples of known HGH levels were mixed and assayed in duplicate. The average recovery was 95.8%.

	Expected Concentration (ng/ml)	Observed Concentration (ng/ml)	% Recovery
1.	2.10	2.13	101.4%
	3.17	2.88	90.9%
	3.44	3.51	102.0%
	0.97	10.5	108.2%
	1.57	1.50	95.5%
	Average Recovery =		***99.6%***
2.	3.70	3.16	85.4%
	9.02	8.38	92.9%
	3.74	5.59	96.0%
	4.94	4.41	89.3%
	Average Recovery =		***90.9%***

Recovery and Linearity Studies

b. Linearity

Two patient samples were serially diluted with the zero standard in a linearity study. The average recovery was 105.1%.

#	Dilution	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
1.	Undiluted	--	66.53	--
	1:2	33.27	32.76	98.5
	1:4	13.63	14.37	105.4
	1:8	8.32	8.49	102.0
	1:16	4.16	4.23	101.7
	1:32	2.08	1.92	92.3
			Avg. =	***100.0%***
2.	Undiluted	--	57.50	--
	1:2	28.75	29.18	101.5
	1:4	14.38	15.00	104.3
	1:8	7.19	7.98	111.0
	1:16	3.59	4.39	122.2
	1:32	1.80	2.01	111.9

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			Avg. =	***110.2%***

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Specificity

The following substances were tested for cross-reactivity:

Hormone Tested	Concentration	Intensity Equivalent to hGH (ng/mL)
HCG (WHO 1st IRP 75/537)	100 mIU/ml 62,000 mIU/ml 500,000 mIU/ml	0 0 0
TSH (WHO 2nd IRP 80/558)	125 µIU/ml 250 µIU/ml 500 µIU/ml	0 0 0
LH (WHO 1st IRP 68/40)	125 mIU/ml 250 mIU/ml 500 mIU/ml	0 0 0
FSH (WHO 2nd IRP HMG)	125 mIU/ml 250 mIU/ml 500 mIU/ml	0 0 0
Prolactin (WHO 1st IRP 75/504)	50 ng/ml 100 ng/ml 500 ng/ml	0 0 0.5

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Hook Effect

No hook effect is observed in this assay at HGH concentrations up to1,000 ng/mL.

Limitations of the Procedure

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with good laboratory practice and adherence to the package insert instructions.
2. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

References / Literature

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