



Human Growth Hormone MICRO-ELISA Test Kit

Prod. No.: T175
Pkg. Size: 96 Tests

Description

The MICRO-ELISA GROWTH HORMONE test is a solid phase sandwich-type enzyme immunoassay (ELISA) Diagnostic Kit for the *in vitro* quantitative determination of human growth hormone (hGH) concentration in human serum.

Summary and Explanation of the Test

Human growth hormone (hGH, somatotropin) is a polypeptide secreted by the anterior pituitary. It is a single chain peptide, 191 amino acids in length, and has a molecular weight of approximately 22,000 daltons. The amino acid sequence of hGH is 85% identical to placenta chorionic somatomammotropin (CS), and 13% identical to prolactin.

Serum hGH measurements are primarily of interest in the diagnosis and treatment of various conditions characterized by forms of abnormal growth. Disorders caused by hyposecretion of growth hormone include dwarfism and failure to attain full growth potential; hypersecretion is associated with gigantism and acromegaly.

The secretion of growth hormone by the pituitary is regulated (by a negative feedback mechanism) by two hypothalamic peptides. Hypothalamic Growth Hormone-Releasing Hormone (GRH, somatotropin) stimulates hGH release, and hypothalamic Somatostatin inhibits hGH release from the pituitary.

Growth Hormone stimulates the production of RNA and, in turn, anabolism. It promotes protein conservation and is engaged in a wide range of mechanisms for protein synthesis. HGH also enhances glucose transport and facilitates glycogen storage. The action of growth hormone is mediated by another family of peptide hormones, the somatomedins. The major serum somatomedins (also called, insulin like growth factors) are IGF-I (Somatomedin-C) and IGF-II.

The half-life of growth hormone in serum is about 20 minutes and its secretion varies throughout the day, therefore, caution must be exercised in the clinical interpretation of growth hormone levels. It is difficult to judge an individual's growth hormone status based on a single serum hGH determination. Many factors are known to influence the rate of growth hormone secretion, including periods of sleep and wakefulness, exercise, stress, hypoglycemia, estrogens, corticosteroid and L-dopa.

Because not all acromegalic individuals have elevated baseline levels of growth hormone, suppression tests based on glucose loading are of value in this context. In spite of the induced hyperglycemia, there is rarely a decrease of baseline levels in acromegaly.

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

A small number of dwarfism cases have been documented in which both the basal level of hGH and the response to challenge testing were normal. Such cases may involve tissue insensitivity to either growth hormone or the somatomedins, or immunoreactivity by biologically inactive growth hormone.

Because of its similarity to prolactin and placental lactogen, earlier growth hormone immunoassay were often plagued with falsely high values in pregnant and lactating women.

The MICRO-ELISA hGH assay provides a rapid, sensitive and reliable test, because there is no cross-reactivity with hCG, TSH, LH, hPL or Prolactin.

Principle of the Procedure

The MICRO-ELISA GROWTH HORMONE test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). Antibody (sheep polyclonal) to hGH is coated to a plastic well (solid phase). Antibody (mouse monoclonal) to hGH is contained in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test serum sample is allowed to react simultaneously with the coated and conjugated antibodies, resulting in the hGH molecule being sandwiched between the solid phase and enzyme-linked antibodies. After a 45 minute incubation at room temperature, the sample well is washed to remove unbound enzyme labeled antibody. An enzyme substrate-chromogen (hydrogen peroxide, H₂O₂, and tetramethylbenzidine, TMB) is added to the well and incubated for 30 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H₂SO₄ stops the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of hGH in the sample.



Reagents

Components in Each 96-Test

Micro-Elisa hGH Diagnostic Kit

1. 96 wells, hGH ANTIBODY COATED **WELLS**: Coated with anti-hGH (rabbit polyclonal); contained in a pack with silica gel desiccant.
2. 1 bottle, 12 ml, hGH ENZYME ANTIBODY **CONJUGATE**: anti-hGH (mouse monoclonal) labeled with horseradish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C red # 40 as coloring agent.
3. 1 bottle, 12 ml, **SUBSTRATE-CHROMOGEN** Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.
4. 1 bottle, 12 ml, **STOP SOLUTION** 1 N H₂SO₄.
5. 1 vial, 1 ml, **0 ng/ml hGH CALIBRATOR/SAMPLE DILUENT**: Bovine serum (Lyophilized); contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
6. 5 vials, 1 ml, hGH **CALIBRATORS**: hGH in bovine serum (Lyophilized); contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; **2.5, 5 10 25 and 50 ng/ml**.

Additional Materials Required

Disposable tip precision pipets - 0.05, 0.1, 0.2 and 1.0 ml.
microtiter plate reader.
Distilled or deionized water.

Storage and Stability

Store all components at 2°-8°C when not in use. The following components may be stored at ambient temperature: **WELLS**, **SUBSTRATE-CHROMOGEN**, **WASH BUFFER** and **STOP SOLUTION**. Expiration date printed on the kit indicates limits of stability.

The hGH ANTIBODY COATED **WELLS** are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2°-30°C.

Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent contamination or deterioration.

Instruments

Performance of the MICRO-ELISA hGH test requires use of a precision microtiter plate reader with a wavelength of 450 ± 20 nm:

Specimen Collection and Preparation

Serum samples are used in the MICRO-ELISA hGH Diagnostic Kit procedure. No special preparation of the patient is necessary; fasting is not required. Repeated

freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

STORAGE: Specimens may be stored in a tightly sealed tube at 2°-8°C for three days. If the serum is not assayed within 3 days, store frozen (-20°C) in a tightly sealed tube for up to 3 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

Micro-Elisa hGH Procedure

Reagent Preparation

Reconstitute each calibrator with 1.0 ml of distilled water. Replace cap and mix. Allow calibrators to stand at room temperature for 30 minutes and mix prior to using. Reconstituted calibrators will be stable until the expiration date stamped on the label when stored at 2-8°C.

Preliminary Comments and Precautions

Patient sample may contain pathogens: treat all samples as potentially infectious.

Reagents contain thimerosal; avoid contact with skin.

Avoid contact with **SUBSTRATE-CHROMOGEN** (tetramethyl-benzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).

CAUTION: Source material used to prepare Calibrators was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. **HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS**. Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

Procedural Notes

1. All test kit components used in an assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18°-30°C) before testing begins.
2. All calibrators, controls, and samples should be tested in duplicate simultaneously. The test samples and controls must be well mixed before use.
3. A separate disposable tip should be used for each sample to avoid cross-contamination. All pipetting steps should be performed with the utmost care and accuracy. Avoid contaminating the reagent pipette tip with the serum sample.
4. The duration of the incubation times must be the same for all wells within a run.
5. Run size should be limited to the number of samples that can be added to antibody coated wells within 10 minutes.



- Samples should be pipetted to the bottom of the antibody-coated wells.
- If microtiter reader is not capable of reading absorbances greater than 2.5, the color should be read after a shorter incubation time with the SUBSTRATE/CHROMOGEN, i.e., 15 minutes.

Test Procedure

- Place sufficient COATED WELLS in a holder to run CALIBRATORS, Quality Control Sera and patient samples in duplicate. Limit run size to the number of samples that can be pipetted in 10 minutes.
- Pipet 50 µl of the CALIBRATORS, Controls or Patient Sample to the corresponding COATED WELL.
- Pipet or dispense 100 µl of the ENZYME ANTIBODY CONJUGATE solution to all the wells and mix gently.
- Incubate at room temperature (18°-30°C) for 45 minutes ± 5 minutes.
- Decant or aspirate and discard liquid contents of all wells. SLAP the inverted wells on a clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
- Fill each well with deionized or distilled water. Fill the wells to overflowing, you cannot cause any carryover between the wells. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.

WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.

- Repeat step 6 three more times (for a total of 4 washes).
- Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
- Pipet or dispense 100 µl (0.1 ml) of SUBSTRATE-CHROMOGEN solution into each well.
- Mix thoroughly and incubate 30 minutes at room temperature (18°-30°C).
- Pipet or dispense 100 µl (0.1 ml) of 1 N H₂SO₄ into each well and mix thoroughly.
- Read the absorbance of each well at 450 ± 20 nm against water.

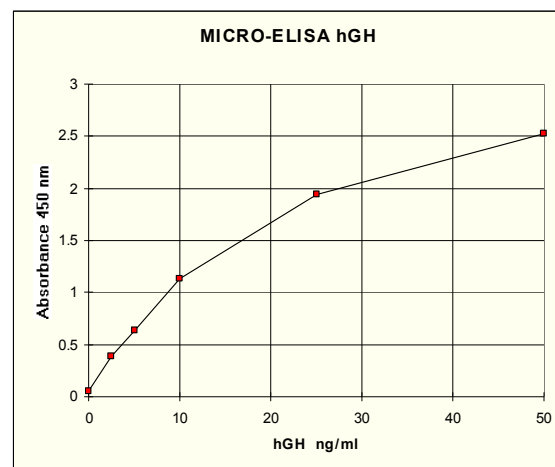
Calculation of Results

- Calculate the mean value for each duplicate sample absorbance. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
- Construct the standard curve by plotting the mean absorbance obtained for each hGH CALIBRATOR on the vertical (Y) axis versus the corresponding hGH concentration on the horizontal (X) axis, using rectilinear graph paper.
- Connect the points with straight-line segments.
- Using the mean absorbance for each sample, read the corresponding hGH concentration in ng/ml from the curve. Multiply the value by the dilution factor if required.

EXAMPLE DATA
DO NOT USE IN PLACE OF CUREVE
DETERMINED AT THE TIME OF ASSAY.

Specimen I.D.	A ₄₅₀	Mean A ₄₅₀	hGH(ng/ml)
CALIBRATOR	0.0 ng/ml	0.050, 0.054	0.052
CALIBRATOR	2.5 ng/ml	0.397, 0.387	0.392
CALIBRATOR	5.0 ng/ml	0.650, 0.633	0.641
CALIBRATOR	10.0 ng/ml	1.125, 1.131	1.128
CALIBRATOR	25.0 ng/ml	1.934, 1.958	1.946
CALIBRATOR	50.0 ng/ml	2.498, 2.561	2.530

SAMPLES	A ₄₅₀	Mean A ₄₅₀	hGH(ng/ml)
# 1 (UNKNOWN #1)	0.428, 0.437	0.432	2.9
# 2 (UNKNOWN #2)	1.015, 1.031	1.023	8.9
# 3 (UNKNOWN #3)	1.674, 1.721	1.697	20.4



The range of this assay is 0 – 50 ng/ml. For specimen with hGH concentrations beyond the standard curve (50 ng/ml), repeat the test by diluting the specimen with the 0 ng/ml Calibrator. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor.



Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls containing azide can not be used. Two controls with normal and elevated values should be used. Pooled human serum or commercially available control sera without azide are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 ng/ml CALIBRATOR exceeds 0.100 it is an indication of careless washing and the assay must be repeated.

Standardization

The MICRO-ELISA hGH CALIBRATORS have been standardized against the World Health Organization International Reference Preparation (1st IS 66/217).

Limitations of the Procedure

As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.

FINAL REACTION STABILITY: The spectrophotometric measurement should be made within 30 minutes after the addition of the H₂SO₄ solution.

Expected Values

HGH values were measured in serum samples from 382 apparently normal individuals and the following results were obtained:

	hGH (ng/ml)
ADULT MALE	0.0-5.0
ADULT FEMALE	0.0-10.0

These values are consistent with those reported in the literature. It is recommended that each laboratory determine its own normal range.

Performance Characteristics of the Test

Assay Specificity

Specificity of this test system was proven by determined a lack of interference of luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH), human chorionic gonadotrophin (hCG), prolactin and human placental lactogen (hPL) when added to serum samples with known growth hormone concentration. The incremental change in growth hormone concentration was measured and the relative cross reactivity calculated by

dividing the change in growth hormone concentration (ng/ml) by the cross reactant concentration (in mIU/ml or ng/ml) times 100. The cross reactivity for LH, FSH, TSH hCG PROLACTIN and hPL were <0.1%

Additionally the specificity of this test system was determined by assaying 50 pregnant female serum samples. All of the female samples assayed for hGH gave results < 4 ng/ml.

Assay Sensitivity

The sensitivity of this assay is defined as the smallest single value that can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty-one replicates at the zero concentration. The calculated sensitivity is <0.5 ng/ml.

Assay Reproducibility

Intra-assay reproducibility was determined by measurement of 9 replicates of three serum pools in a single run.

	Mean hGH (ng/ml)	SD	%CV
Serum A	1.38	0.12	8.47
Serum B	3.71	0.16	4.39
Serum C	9.72	0.55	5.65

The interassay reproducibility was determined by duplicate measurement of three serum pools in nine separate runs.

	Mean hGH (ng/ml)	SD	% CV
Serum A	1.58	0.10	6.47
Serum B	4.52	0.13	2.94
Serum C	10.04	0.31	3.12

Assay Linearity

A study was performed diluting a serum sample containing an elevated level of hGH with the 0 ng/ml CALIBRATOR to determine the linearity of the MICRO-ELISA hGH.

Dilution Factor	hGH ng/ml		% of Expected Value
	Expected Value	Observed Value	
undiluted	-	24.54	-
3:4	18.41	20.25	110 %
1:2	12.27	11.40	93 %
1:4	6.14	5.96	97 %
1:8	3.07	3.25	106 %

Assay Recovery

Three aliquots of human sera with a high hGH concentration of 4.50 ng/ml were spiked with 3.25, 10.29 and 20.77 ng/ml of hGH. The samples were assayed in duplicate.

Added hGH	hGH ng/ml		% Recovery
	Expected Value	Measured Value	
0.0	4.50		
3.25	7.65	8.34	109 %
10.29	14.79	13.80	93 %
20.77	25.27	27.61	109 %



Comparison to Other hGH Tests

Correlation studies on a random group of 67 serum samples with a range of values from <0.3 – 30 ng/ml, were performed using the quantitative results from the MICRO-ELISA hGH Test and THE Hybritech Tandem-R hCH test. The correlation coefficient of the test results was 0.927.

	<u>Slope</u>	<u>Y-Intercept</u>	<u>Correlation Coefficient</u>
n= 67	0.945	-0.03	0.927

References

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Warranty

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