Enzyme Immunoassay for the Quantitative Determination of Human Growth Hormone (HGH) Concentration in Human Serum

Catalog Number: 10330

FOR RESEARCH USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

HGH Enzyme Immunoassay

INTENDED USE

For the quantitative determination of the Human Growth Hormone (HGH) concentration in human serum.

INTRODUCTION

Human growth hormone (HGH, somatotropin) is a polypeptide secreted by the anterior pituitary. It has 191 amino acids in length and has a molecular mass of approximately 22,000 daltons. Its metabolic effects are primarily anabolic. 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. 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, and hypersecretion is associated with gigantism and acromegaly.

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 growth hormone immunoassays were often plagued with falsely high values in pregnant and lactating women.

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 from 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. Propanolol 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 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.

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

PRINCIPLE OF THE TEST

The HGH Quantitative Test Kit 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 Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, and the color is changed to yellow and measured spectrophotometrically at 450nm. The concentration of HGH is directly proportional to the color intensity of the test sample.

REAGENTS

Materials provided with the kit:

- Sheep Anti-HGH-coated microtiter plate with 96 wells.
- Reference standard set, containing 0, 2.5, 5, 10, 25, and 50 ng/ml HGH, ready to use.
- Enzyme Conjugate Reagent, 13 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes: 50 µl, 100 µl and 1.0 ml.
- Distilled water.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater.
- Graph paper.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

Storage of Test Kit and Instrumentation

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with ***distilled or deionized water***. ***(Please do not use tap water.)***
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl TMB Reagent solution into each well. Gently mix for 10 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. ***It is important to make sure that all the blue color changes to yellow color completely.***
13. Read the optical density at 450 nm with a microtiter plate reader ***within 15 minutes***.

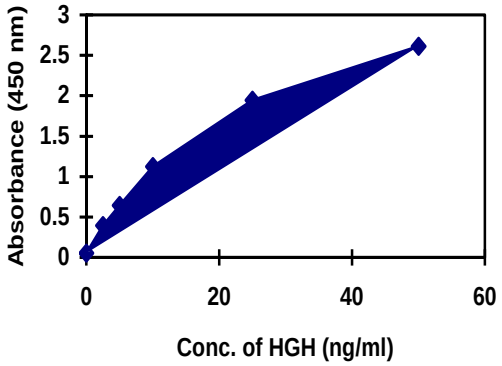
Calculation of Results

1. Calculate the average absorbance values (A_{450}) for each set of reference standards, control, 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.

Example of Standard Curve

Results of a typical standard run with optical density readings at 450 nm shown in the Y-axis against HGH concentrations shown in the X-axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

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



Expected Values and Sensitivity

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. 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 give a more accurate assessment of pituitary dysfunction. Confirmation of diagnosis requires provocative tests, either stimulation or suppression.

The minimal sensitivity of the test is 0.5 ng/ml.

Limitations of the Procedure

Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

References

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