Monobind Inc.
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AccuLite
CLIA Microwells

Human Growth Hormone (hGH) Test System

Product Code: 1775-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Growth Hormone Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Growth hormone (hGH, somatotropin), secreted from the anterior pituitary, is a polypeptide with two intra-chain disulfide bridges, which circulates free or bound to number of different GH-binding proteins. Several forms of growth hormone have been identified[1] with the major being of molecular weight 22,000 daltons containing 191 amino acid residues. A 20,000-dalton variant, which posses all known biological functions of GH, has also been demonstrated to be important. The primary biological actions of the hormone are in direct growth promoting. GH exerts its effect directly on target organs such as bones and muscles and indirectly through the release of somatomedins, a family of insulin-like growth factor (IGF) hormones, produced in the liver.[2] In particular, somatotropin C (IGF-1) is essential for bone growth during childhood.

The clinical usefulness of the measurement of growth hormone (GH) in children has been well established in ascertaining linear bone growth along the epiphyseal plate. Abnormal elevated levels lead to gigantism while complete absence slows the rate of growth to one-third to one-half of normal. In adults, the epiphyseal growth plates have fused; GH excess gradually produces acromegaly, a coarse thickening of the bones of the skull, hands and feet.

In this method, GH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of GH) are added and the reactants mixed. Reaction between the various GH antibodies and native GH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-growth hormone antibody bound conjugate is separated from the unbound enzyme-growth hormone conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce light.

The employment of several serum references of known growth hormone levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with growth hormone concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (Type 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-Troponin-I antibody.

Upon mixing biotin labeled monoclonal antibody, the enzyme-labeled antibody and a serum containing the native antigen reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(x\text{-}GH)} + Ag_{GH} + {}^{Btn}Ab_{(m)} \underset{k_a}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(x\text{-}GH)} - Ag_{GH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{GH} = Native Antigen (Variable Quantity)
$^{ENZ}Ab_{(x\text{-}GH)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{ENZ}Ab_{(x\text{-}GH)} - Ag_{GH} - {}^{Btn}Ab_{(m)}$ = Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(m)} - Ag_{cTnI} - {}^{Btn}Ab_{(m)} + \underline{Strept_{CW}} \Rightarrow$ <u>immobilized complex</u>
$\underline{Strep_{CW}}$ = Streptavidin immobilized on well
<u>Immobilized complex</u> = sandwich complex bound to the solid surface

After sufficient time for reaction, the antibody bound fraction is separated from unbound antigen by decantation or aspiration. 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 can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Growth Hormone Calibrators --1ml/vial - Icons A-F
Six (6) vials of references for hGH Antigen in human serum at levels of 0(**A**), 2(**B**), 10(**C**), 25(**D**), 50(**E**) and 150(**F**) µIU/ml. Store at 2-8°C. A preservative has been added.

Note: Calibrated against the 1st IS WHO 80/505. To convert to mass units in terms of the International Standard WHO 2nd IS# 98/574 divide by 3.7. *WHO 80-505 (*µIU/ml) / 3.7 = *WHO 98/574 (ng/ml)*

B. hGH Tracer Reagent —13ml/vial - Icon Ⓔ
One (1) vial contains horseradish peroxidase (HRP), labeled affinity purified antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Light Reaction Wells – 96 wells – Icon ⇓
One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial contains a surfactant dissolved in buffered saline. A preservative has been added. Store at 2-8°C.

E. Signal Reagent A – 7.0ml/vial – Icon C[A]
One (1) vial contains luminol in buffer. Store at 2-8°C.

F. Signal Reagent B – 7.0ml/vial – Icon C[B]
One (1) vial contains hydrogen peroxide (H_2O_2) dissolved in buffer. Store at 2-8°C.

G. Product Insert

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Pipette(s) capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Luminometer
5. Container(s) for mixing of reagents (see below).
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Storage container for storage of wash buffer.
11. Distilled or deionized water.
12. Quality Control Materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS.

Safe Disposal of kit components must be according to local and regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium 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.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2 - 8°C.
   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional.***

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of hGH Tracer Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 45 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
8. Add 0.100 ml (100µl) of working signal reagent to all wells (see Reagent Preparation Section).). **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION
9. Incubate for five (5) minutes in the dark.
10. Read the relative light units in each well for 0.2 – 1.0 seconds. **The results should be read within thirty (30) minutes of adding the signal solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of human growth hormone in unknown specimens.

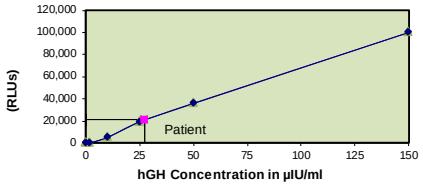
1. Record the RLUs obtained from the printout of the microplate luminometer as outlined in Example 1.
2. Plot the light intensity for each duplicate serum reference versus the corresponding hGH concentration in µg/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of hGH for an unknown, locate the average RLUs of the 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). In the following example, the average RLUs (20937) of patient intersects the calibration curve at (27.5µIU/ml) hGH concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (µIU/ml)
Cal A	A1	10	10	0
	B1	9		
Cal B	C1	274	274	2.0
	D1	274		
Cal C	E1	5146	5215	10.0
	F1	5284		
Cal D	G1	18854	19228	25.0
	H1	19602		
Cal E	A2	35865	36073	50.0
	B2	36282		
Cal F	C2	101083	100000	150.0
	D2	98917		
Ctrl 1	E2	5782	5706	***10.5***
	F2	5630		
Ctrl 2	G2	46478	46981	***62.9***
	H2	47484		
Patient	A3	20812	20937	***27.5***
	B3	21062		

Figure 1



* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion eliminates differences cause by efficiency of the various instruments that can be used to measure light output. The conversion varies between instruments and should be established for each instrument before using it as a factor.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. This immunoassay has been designed so that the high dose "hook effect" is not an issue for elevated samples. Specimens with concentrations greater than 150µIU/ml should be diluted and re-assayed.
9. Patients on hGH replacement may develop antibodies to hGH that may interfere in the assay and cause falsely low values. Genetic variants or degradation products may alter antibody-binding characteristics and affect final results. Such samples may display discordant results on different assays that utilized antibodies, which recognize different epitopes.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Growth hormone secretion follows a circadian rhythm characterized by discontinuous pulsatile discharge bursts with intervening periods during the day when GH levels are undetectable. The highest levels, in two major bursts, are usually attained within one or two hours after the onset of sleep. Other physiological stimuli of growth hormone are stress, exercise, high protein meals and hypoglycemia.
8. Hyperglycemia inhibits growth hormone secretion. Age is an important factor in growth hormone concentrations. At birth, GH is high and generally declines with age with the exception of a burst during the growth phase of adolescence. Women typically have a 50% higher level than their age-matched males.
9. Since growth hormone concentration is pulsatile and sporadic during the course of the day (coupled with its' short half- life), single serum random levels do not yield clinically useful information. To overcome this problem, provocative tests are utilized that employ physiological or pharmacological stimuli to induce the secretion or inhibition of GH. For these reasons, the determination of growth hormone alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF 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. The well rested, fasting (12 hours) subjects should have hGH values of 60 µIU/ml or less.

With this caveat in mind, 45 apparent healthy adults were assayed the hGH immunoassay. The results are depicted in Table 1.

TABLE I
Expected Values (in µIU/ml)

	N	Mean	Range
Specimens	45	10.4	0 - 53

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 GH release: exercise,[3] L-dopa administration,[4] insulin tolerance test,[5] and arginine infusion.[6] Each laboratory should assess the normal response, but a peak GH 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.

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the hGH AccuLite® CLIA Test System were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in µIU/ml)

Sample	N	X	σ	C.V.
Level 1	24	12.57	0.30	2.4
Level 2	24	28.73	0.78	2.7
Level 3	24	62.79	1.55	2.5

TABLE 3
Between Assay Precision* (Values in µIU/ml)

Sample	N	X	σ	C.V.
Level 1	14	10.54	1.11	10.5
Level 2	14	23.99	1.20	5.0
Level 3	14	55.04	3.23	5.9

*As measured in several experiments in duplicate.

14.2 Sensitivity

The hGH AccuLite® CLIA test system has a sensitivity of 0.006µIU/well. This is equivalent to a sample containing 0.118µIU/ml GH concentration. The sensitivity was ascertained by determining the variability of the 0 ng/ml calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This method was compared with a reference method. Biological specimens from normal and elevated samples were assayed. The total number of such specimens was 65. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	14.5	y= 1.03 (x) + 0.121	0.975
Reference (x)	14.1		

Only slight amounts of bias between the hGH AccuLite® CLIA test system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross reactivity of the hGH AccuLite® CLIA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Growth Hormone needed to produce the same absorbance.

Substance	Cross Reactivity
Growth Hormone (GH)	1.0000
Luteinizing Hormone (LH)	< 0.0001
Follicle Stimulating Hormone (FSH)	< 0.0001
Chorionic gonadotropin (CG)	< 0.0001
Thyroid Stimulating Hormone (TSH)	< 0.0001
Prolactin Hormone (PRL)	< 0.0001

15.0 REFERENCES

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MP1775 **Product Code: *1775-300***

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)

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(EN 980/ISO 15223)