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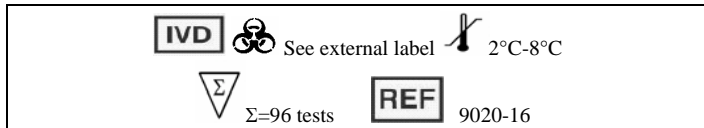
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HUMAN GROWTH HORMONE

Chemiluminescence Immunoassay

CAT No: 9020-16

INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).

ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.

The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the **MPL2** microplate luminometer from Berthold Detection System, **Lus2** microplate luminometer from Anthos, **Centro LB960** microplate luminometer from Berthold Technologies, and **Platelumino** From Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

INTRODUCTION OF HGH IMMUNOASSAY

Human growth hormone (HGH, somatotropin) is a polypeptide secreted by the anterior pituitary. It is 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 response 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, HGH and prolactin.

PRINCIPLE OF THE TEST

The assay system utilizes one monoclonal anti-HGH antibody for solid phase (microtiter wells) immobilization and another monoclonal anti-HGH antibody in the antibody-enzyme (horseradish

peroxidase) conjugate solution. The standards and test specimen (serum) are added to the HGH antibody coated microtiter wells. Then HGH antibody labeled with horseradish peroxidase (conjugate) is added. If human HGH is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the HGH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 1 hour incubation at Room Temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in the Berthold Detection Systems MPL2 Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of HGH antigen in the sample. By reference to a series of HGH standards assayed in the same way, the concentration of HGH in the unknown sample is quantified.

MATERIALS AND COMPONENTS

Materials Provided with Test Kit

1. Anti-HGH antibody coated microtiter plate, 96 wells.
2. Enzyme conjugate reagent, 12 ml.
3. HGH reference standards containing 0, 1.0, 2.5, 7.5, 15, and 30 ng/ml (WHO, 1st IRP, 66/217) HGH. Liquid, ready for use. 1 set.
4. 20x Wash Buffer, 30 ml
5. Chemiluminescence Reagent A, 6.0 ml
6. Chemiluminescence Reagent B, 6.0 ml

Materials Required but not Provided

1. Distilled water.
2. Precision pipettes: 0.05ml, 0.1ml, 0.2ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Reagent A and B.
5. Microtiter well reader.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

REAGENT PREPARATION

1. To prepare substrate solution, make an 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
2. Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 50 µl of HGH standards, specimens, and controls into the appropriate wells. Gently but thoroughly mix for 10 seconds.
2. Dispense 100 µl of enzyme conjugate reagent into each well. Mix gently for 30 seconds. It is very important to have complete mixing in this setup. Incubate at Room Temperature for 1 hours.
3. Remove the incubation mixture by emptying the plate content into a waste container.
4. Rinse and flick the microtiter wells 5 times with washing buffer.
5. Strike the wells sharply onto absorbent paper to remove residual water droplets.
6. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.

7. Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

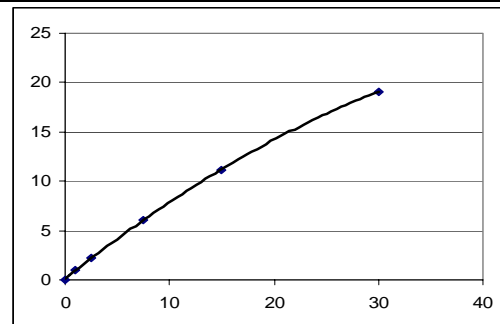
CALCULATION OF RESULTS

1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend to use a proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regression or cubic spline regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against HGH concentration in Ng/ml on linear graph paper, with RLU 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 are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

HGH (ng/ml)	Relative Light Units (RLU) (10 ⁵)
0	0.01
1	0.98
2.5	2.21
7.5	6.11
15	11.19
30	19.11



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

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

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