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Cat # 9021-16

**CHEMILUMINESCENCE**  
ENZYME IMMUNOASSAY (CLIA)  
Human Insulin

# Insulin

Cat # 9021-16

### Chemiluminescence Enzyme Immunoassay for the Quantitative Determination of Human Insulin Concentrations in Human Serum

#### 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, INSULIN, 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.

## SUMMARY AND EXPLANATION OF THE TEST

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized in the  $\beta$ -cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form Insulin and insulin. Both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain and B chain (21 and 30 amino acids respectively). The two chains are linked together by two inter-chain disulphide bridges. There is also an intra-chain disulphide bridge in the A chain.

Secretion of insulin is mainly controlled by plasma glucose concentration, and the hormone has a number of important metabolic actions. 1st principal function is to control the uptake and utilization of glucose in peripheral tissues via the glucose transporter. This and other hypoglycemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycemic hormones including glycogen, epinephrine (adrenaline), growth hormone and cortisol.

Insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM) and some other conditions such as hypopituitarism. Insulin levels are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushion's syndrome and acromegaly.

The Insulin Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-Insulin antibody for solid phase (microtiter wells) immobilization and another anti-Insulin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the Insulin antibody coated microtiter wells. Then anti-Insulin antibody labeled with horseradish peroxidase (conjugate) is added. If human Insulin is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the Insulin 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 a Luminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of INSULIN in the sample. By reference to a series of INSULIN standards assayed in the same way, the concentration of INSULIN in the unknown sample is quantified.

## PRECAUTIONS

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 required tests. 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

## 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 red-top venipuncture tube without additives. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Insulin is not stable in serum basis. Samples should be used as fresh as possible. Refrigerated at 2-8°C for a maximum period of one day only. 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 repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

## MATERIALS AND COMPONENTS

### Materials provided with the test kits:

- Monoclonal anti Insulin antibody coated microtiter plate with 96 wells.
- Enzyme conjugate reagent, 12 ml.
- Insulin reference standards containing; 0, 5, 25, 50, 100, and 200 uIU/ml. lyophilized. 0.5mlx2sets.
- Wash Solution Concentrate, 50X, 15ml
- Chemiluminescence Reagent A, 6.0 ml.
- Chemiluminescence Reagent B, 6.0 ml.

The standard calibrators were calibrated using a reference preparation, which was assayed against the WHO 1st IRR 66/304

### Materials required but not provided:

- Precision pipettes and tips, 0.05 ml, 0.1 ml,
- Disposable pipette tips.
- Distilled water.
- Vortex mixer.
- Absorbent paper or paper towel.
- Microtiter plate reader.
- Graph paper.
- Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5% (optional).
- Adjustable volume (200-1000µl) repeat dispenser.
- Container(s) for mixing of reagents (see below).
- Timer.
- Quality Control Materials.

## REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do NOT induce foaming.
2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.
3. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-8°C.

## ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **50**µl of Insulin 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 60 minutes.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with 1 x wash buffer (300µl each well). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
4. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
5. Read wells with a chemiluminescence microwell reader 5 minutes later. (Between 5 and 20 min. after dispensing the substrates).

### Important Note:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bobbles existing in the wells, the false readings will be created. Please use distilled water to remove the bobbles before adding the substrate.

## 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. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against INSULIN concentration in uIU/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 INSULIN in uIU/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.

Insulin Values (uIU/ml)	Relative Light Units (RLU) (10 <sup>4</sup> )
0	3
5	6
25	20
50	39
100	70
200	124

## LIMITATIONS OF PROCEDURE

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the

stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction.

5. Plate readers measure vertically. Do not touch the bottom of the wells.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Highly lipemic, hemolysed or grossly contaminated specimen(s) should not be used.
8. Patient samples with Insulin concentrations above 200 uIU/ml may be diluted with the zero calibrator and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
9. Use components from the same lot. No intermixing of reagents from different batches.

## EXPECTED VALUES

Insulin values are consistently higher in plasma than in serum; thus, serum is preferred. Compared with fasting values in non-obese nondiabetic individuals, insulin levels are higher in obese non-diabetic subjects and lower in trained athletes.

Each laboratory is advised to establish its own ranges for normal and abnormal populations. These ranges are always dependent upon locale, population, laboratory, technique and specificity of the method.

Based on the clinical data gathered by Monobind in concordance with the published literature the following ranges have been assigned.

These ranges should be used as guidelines only:

Children < 12 yrs	< 10 µIU/ml
Adult (Normal)	0.7 – 9.0 µIU/ml
Diabetic (Type II)	0.7 – 25 µIU/ml

## Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 uIU/ml serum calibrator and using the **2SD** (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 2.0 uIU/ml.

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