# HUMAN INSULIN

**ENZYME IMMUNOASSAY TEST KIT**
**Catalog Number: 10801**

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

## INTENDED USE

The Quantitative Determination of Insulin Concentrations in Human Serum

## INTRODUCTION

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized in the β-cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form Insulinand 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 disulphride 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.

## TEST PRINCIPLE

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 Insulinis 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 to remove unbound labeled antibodies. A solution of TMB 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. The color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of Insulin is directly proportional to the color intensity of the test sample.

## 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:**

- Antibody coated microtiter plate 96 wells
- Enzyme Conjugate Reagent 12 mL
- TMB Substrate 12 mL
- Stop Solution 12 mL
- Wash Concentrate (50X) 15mL
- Insulin reference standards, containing 0, 5, 25, 50, 100, and 200 µIU/mL, in liquid form (ready to use) or lyophilized form the standard calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRR 66/304)

**Materials required but not provided:**

- Precision pipettes, 40-200 µL, 200-1000 µL
- Disposable pipette tips
- Distilled water
- Vortex mixer
- Absorbent paper or paper towel
- Microtiter plate reader
- Graph paper
- Microplate Reader with 450nm and 620nm wavelength absorbance capability (The 620nm filter is optional)
- 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. Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 50x wash concentrate to a final volume of 750mL.

3. If reference standards are lyophilized, reconstitute each standard with 0.5 mL distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.

## ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **50**µL of Insulinstandards, 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 a complete mixing in this step. 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 washing 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 of TMB substrate reagent into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20 minutes.

5. Stop the reaction by adding **100**µL of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
6. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

## IMPORTANT NOTE

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

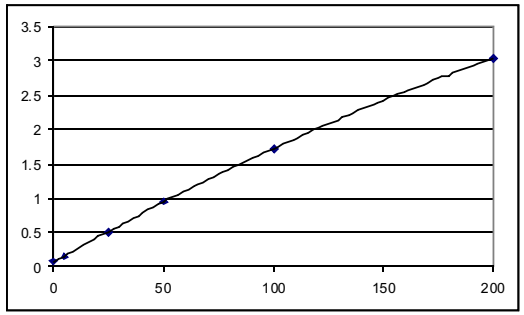
## CALCULATION OF RESULTS

Calculate the mean absorbance value for each set of Insulinreference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in units per mL on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of Insulin in units per mL from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

## EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density reading at 450nm shown in the Y axis against Insulin concentrations shown in the X axis.

| Insulin Values (µIU/mL) | Absorbance (450nm) |
|---|---|
| 0 | 0.075 |
| 5 | 0.148 |
| 25 | 0.497 |
| 50 | 0.955 |
| 100 | 1.716 |
| 200 | 3.039 |




## 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 lipemeic, hemolysed or grossly contaminated specimen(s) should not be used.
8. Patient samples with Insulin concentrations above 200 µIU/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 µU/mL |
| Diabetic (Type II) | 0.7-25 µIU/mL |

## SENSITIVITY

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

## REFERENCES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7/2014


**Chemux Bioscience,Inc.**

**Website:** www.chemux.com

385 Oyster Point Blvd Suite5-6.,South San Francisco,CA94080
Tel:+1-650-872-1800