Insulin

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for Quantitative Determination of Human Insulin (INSL) Concentrations in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Insulin test is intended for the Quantitative Determination of Insulin Concentrations in Human Serum. For In Vitro Diagnostic Use Only.

INTRODUCTION

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

PRINCIPLE OF THE ASSAY

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 incubation, the wells are washed & bound enzyme is detected by adding substrate. The reaction is stopped after specified time with stop solution and absorbance is determined for each well using an ELISA reader. The concentration of Insulin is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with monoclonal anti- Insulin antibody.
- Insulin Enzyme Conjugate. Ready to use.
- TMB Substrate. Ready to use
- Stop Solution. Ready to use
- Insulin Standard set of 6 standards labeled as A to F in Lyophilized form. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).
- Control Set in lyophilized form. For control range refer vial label.
- Pack Insert
- Plate Sealers
- Protocol Sheet
- Microwell Holder

Materials required but not provided

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **Insulin** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to pink at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable for upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Only fasting morning fresh serum samples to be used for testing.
2. Samples should be assayed as fresh as possible, If the samples cannot be assayed on the same day when collected, then the samples may be stored at -20°C for up to 30 days.
3. Collect Blood specimen by venipuncture according to standards procedure.
4. Only serum should be used.
5. Avoid grossly hemolytic, lipemic or turbid samples.
6. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
7. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
8. Do not heat inactivate before use.
9. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
10. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

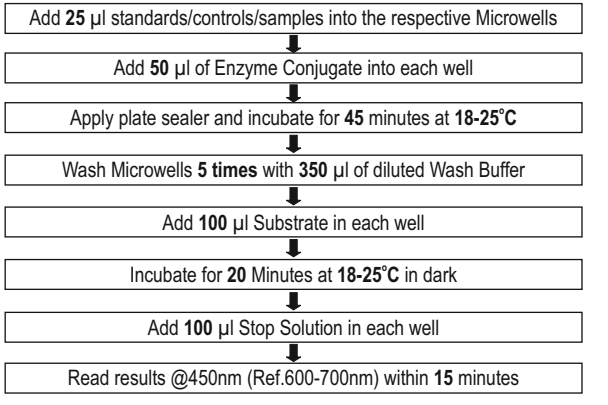
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens, standards and controls should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

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 Wash Buffer **20 times** (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
3. If reference standards and controls are lyophilized, reconstitute each standard and control with **0.5 ml** distilled water. Allow the reconstituted material to stand for at least **20 minutes**. Reconstituted standards and controls should be sealed and stored at 2-8°C.

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **25** µl of standards, controls and sera into the appropriate wells.
2. Dispense **50** µl of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for **45 minutes**.
3. After incubation, empty the microtitre wells and wash the plate **5 times** with **350** µl of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
4. Dispense **100** µl of TMB substrate into each well. Incubate at room temperature (18-25°C), 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 450/630 nm with a microtiter plate reader within **15 minutes**.



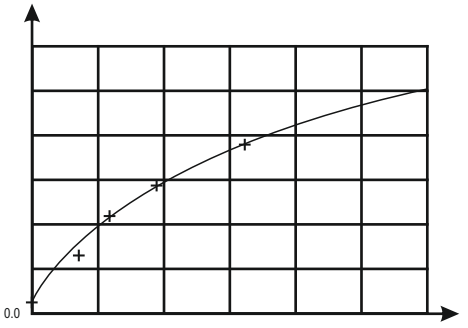
CALCULATION OF RESULTS

Construct a standard curve by plotting the absorbance obtained from each reference standard against its concentration in µIU/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of Insulin in µIU/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 (ref 600 – 700nm) shown in the Y axis against Insulin concentrations shown in the X axis.

Insulin (µIU/ml)	Absorbance (450nm)
A	0.011
B	0.133
C	0.450
D	0.766
E	1.563
F	1.849



This Standard curve is for the purpose of illustration only, and should not be used to calculate samples. Each user should obtain his or her own standards curve and data.

Expected Ranges of values

Insulin values are consistently higher in plasma than in serum; thus, serum is preferred. Compared with fasting values in non-obese non diabetic 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 local population, laboratory, technique and specificity of the method. Based on the clinical data gathered 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

The minimum detectable concentration of Insulin by this assay is estimated to be 2.0 µIU/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal Study **Insulin** was evaluated against commercially available licensed kit with 90 random clinical samples and **Insulin** has demonstrated 100% clinical correlation with the commercially available licensed kit.
2. Precision: **Insulin** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with **Insulin**	Coefficient of Variation (CV)
Level 1	10	9.70	5.25
Level 2	10	44.51	4.89
Level 3	10	147.38	5.51

B) External Evaluation:

Insulin ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Insulin** ELISA has demonstrated 100% correlation with the reference method.

*Data file: Orchid Biomedical Systems (P) Ltd.

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards, controls and samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

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6. Data on file: Orchid Biomedical Systems (P) Ltd.

SYMBOL KEYS

	Temperature Limitation		Consult Instructions for use
	Manufacturer	IVD	*In vitro* Diagnostic Medical Device
	Use by	REF	Catalogue Number
	Date of Manufacture	LOT	Batch Number / Lot Number
	This side up	Σ	Contains sufficient for <n> tests
2	Do not reuse		

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