Human Insulin ELISA Kit

Human Insulin ELISA Kit is an Enzyme Immunoassay kit for the quantification of Human Insulin in serum and plasma (heparin, EDTA).

Catalog number: ARG82313

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

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INTRODUCTION

Insulin is a peptide hormone produced by beta cells of the pancreatic islets; it is considered to be the main anabolic hormone of the body. It regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of glucose from the blood into liver, fat and skeletal muscle cells. In these tissues the absorbed glucose is converted into either glycogen via glycogenesis or fats (triglycerides) via lipogenesis, or, in the case of the liver, into both. Glucose production and secretion by the liver is strongly inhibited by high concentrations of insulin in the blood. Circulating insulin also affects the synthesis of proteins in a wide variety of tissues. It is therefore an anabolic hormone, promoting the conversion of small molecules in the blood into large molecules inside the cells. Low insulin levels in the blood have the opposite effect by promoting widespread catabolism, especially of reserve body fat. [Provide by Wikipedia: Insulin]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for Human Insulin has been pre-coated onto a 96 well microplate. Antibody-conjugate specific for Human Insulin and Standards or samples are pipetted into the wells and any Human Insulin present is bound by the immobilized antibody and Antibody-conjugate to form an Antibody-Antigen-Antibody complex. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of Insulin bound in the initial step. The color development is stopped

by the addition of stop solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of Insulin in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strip	4°C
Standard, 0 mU/L (lyophilized)	1 vial	4°C
Standard, 6 mU/L (lyophilized)	1 vial	4°C
Standard, 30 mU/L (lyophilized)	1 vial	4°C
Standard, 110 mU/L (lyophilized)	1 vial	4°C
Standard, 250 mU/L (lyophilized)	1 vial	4°C
Antibody Conjugate	1 mL	4°C
Conjugate Diluent	11 mL	4°C
Low Control (lyophilized)	1 vial	4°C
High Control (lyophilized)	1 vial	4°C
30X Wash Buffer	50 mL	4°C
TMB Substrate	15 mL (ready to use)	4°C (protect from light)

Stop Solution	15 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm
- Centrifuge and centrifuge tube
- Vortex or mixer
- Deionized or distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Return any unused microplate strips to the plate pouch with desiccant.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- The reagent preparation method might be different from lot to lot, so please check the lot and follow the instructions given in this manual.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 30X Wash Buffer, warm to 37°C until the

crystals are completely dissolved.

- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 1,000 x g for 15 minutes at 4°C.

Plasma: Collect blood with EDTA or citrate and centrifuge at 1,000 x g for 15 minutes at 4°C.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.

2. Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.
3. Store all samples on ice after preparation and use immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles. (no more than twice)

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 30X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 25 mL of 30X Wash Buffer into 725 mL of distilled water to a final volume of 750 mL)
- Working Antibody Conjugate: Transfer the entire contents of the vial containing Antibody Conjugate (1.0 mL) into the bottle of Conjugate Diluent (11.0 mL) and mix thoroughly.
- Controls: Reconstitute each of controls by the addition of 1.0 mL of distilled water. Allow these to stand for 5 minutes, then mix gently to ensure all solids are dissolved. Stability of the reconstituted Controls is two weeks when stored at 2-8°C.
- Standards: Reconstitute each of the standards by the addition of 1.0 mL of distilled water. Allow these to stand for 5 minutes, then mix gently to ensure all solids are dissolved. Stability of the reconstituted Standards and Controls is two weeks when stored at 2-8°C.

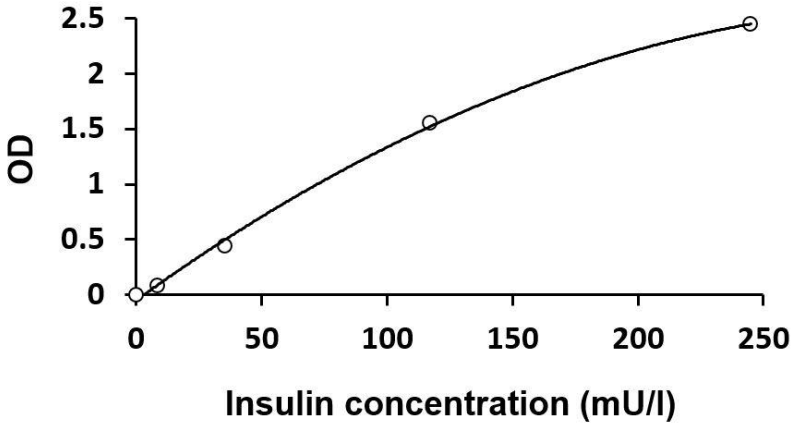
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **100 µL** of **Working Antibody Conjugate** to the **Antibody Coated Microplate**.
2. Add **25 µL** of **each Standard or sample** into respective wells. Cover the plate and incubate for **2 hours** at **37°C.**
3. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with **1X Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **TMB Substrate** to each well. Incubate for **15 minutes** at **room temperature** in the dark.
5. Immediately Add **100 µL** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
6. Read the absorbance at **O.D. 450 nm**. If available, with the optical density normalized by subtraction of the **O.D. 620/650 nm**.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human Insulin ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Subtract zero point (S0) from all standards and unknowns to determine corrected absorbance.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter

Logistics is the preferred method. Other data reduction functions may give slightly different results.

4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. Samples with concentrations higher than that of the highest standard should be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

QUALITY ASSURANCE

Sensitivity

0.9 mU/L

Cross Reactivity

Cross reactivities of related proteins were investigated at concentrations of 100 pmol/L Results are expressed as percentages of the reactivity of an identical concentration of Insulin.

Peptide	Cross Reactivity (%)
Insulin	100
Glargine	104
Aspart	94
Lispro	108
Proinsulin	1.2
C-peptide	0