Human Insulin ELISA Kit

Enzyme Immunoassay for the quantification of human Insulin in serum and plasma

Catalog number: ARG80488

Package: 96 wells

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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 Cpeptide 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. Its principal function is to control the uptake and utilisation of glucose in peripheral tissues via the glucose transporter. This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycaemic hormones including glucagon, 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 Cushing's syndrome and acromegaly.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Insulin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Insulin present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Insulin is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) 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 acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Insulin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Zero Standard	1 X 3 ml (Ready-to-use)	4°C
Standards 1-5 (6.25, 12.5, 25, 50, 100 µIU/ml)	5 X 1 ml (Ready-to-use)	4°C
Biotin-Conjugated Antibody	5 ml (Ready-to-use)	4°C
HRP-Streptavidin Complex	7 ml (Ready-to-use)	4°C
40X Wash buffer	30 ml	4°C
TMB substrate reagent	14 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	14 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (Optional: 620 nm as reference wavelength)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. Opened reagents must be stored at 2-8 °C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 8 weeks if stored as described above.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Bring all reagents and required number of strips to room temperature prior to use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents must be mixed without foaming before use.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- Once the test has been started, all steps should be completed without interruption.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.
b) Samples containing sodium azide should not be used in the assay.
c) Specimens should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying. Specimens held for a longer time (at least one year) should be frozen only once at -20°C prior to assay.
d) Thawed samples should be inverted several times prior to testing.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. E.g. Add 30 ml of 40 X Wash buffer into 1170 ml of distilled water to a final volume of 1200 ml. The diluted 1X Wash buffer is stable for 2 weeks at room temperature.
- Samples: In an initial assay, if a specimen is found to contain more than the highest standard, the specimens can be diluted with Zero standard and re-assay. For the calculation of concentration this dilution factor has to be taken into account.

Example:

a) Dilution 1:10: 10 µL sample + 90 µL Zero Standard (mix thoroughly)

b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Zero Standard (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **25 µl** of **standards, controls and samples** in duplicate into wells.
3. Add **25 µl** of **Biotin-conjugated antibody** into each well. Mix thoroughly for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate the plate for **30 minutes at RT.**
5. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer** (300-400 µl) using a squirt bottle, manifold dispenser, or autowasher. 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.
6. Add **50 µl** of **HRP-Streptavidin complex** to each well. Cover wells and incubate for **30 mins at RT**.
7. Aspirate each well and **wash as step 5.**
8. Add **50 µl** of **TMB substrate reagent** to each well. Incubate for **15 minutes at room temperature in dark.**
9. Add **50 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at **450 nm** (and reference filter **620 nm**) immediately. It is recommended reading the absorbance within 10 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using 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. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. 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.

5. 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)

6. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 100 µIU/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

7. EXPECTED NORMAL VALUES: It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the Insulin ELISA the following values are observed: 2 µIU/ml to 25 µIU/ml

The results alone should not be the only reason for any therapeutic

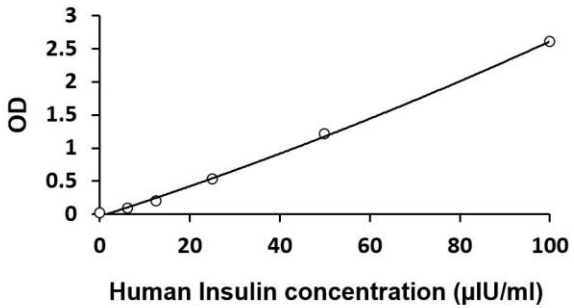
consequences. The results should be correlated to other clinical observations and diagnostic tests.

8. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	0	1	2	3	4	5
Insulin (µIU/ml)	0	6.25	12.5	25	50	100
Insulin (ng/mL)	0	0.27	0.542	1.083	2.165	4.33
Conversion	Insulin (µIU/ml)x **0.0433** = Insulin (ng/mL)					

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity of the Insulin ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Standard A and was found to be 1.76 µIU/ml.

Specificity

Porcine Insulin (>100%)

Bovine Insulin (>100%)

Dog Insulin (82%)

Rabbit Insulin (63%)

Rat Insulin (0%)

Human Proinsulin (0%)

Porcine Proinsulin (0%)

Bovine Proinsulin (0%)

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2.2% and inter-assay precision was 4.45%.

Recovery

91.8-109.6%

Linearity

88.4-110.4%