Human Insulin ELISA Kit

to quantitatively determine Human Insulin in Human Blood

INSTRUCTION MANUAL

FOR ELISA KIT No: LT17626ETKKBA

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No.44,
Pitampura, Delhi-110034, India
Toll Free: 1800-120-2434
Mobile: +91-98105-21400 | Fax: +91-11-42208444
customerservice@lifetechindia.com

INTENDED USE

This kit is used to quantitatively determine the Human Insulin in the sample of Human Blood Serum or Plasma. For *in vitro* use only.

MANUAL VERSION 1.02X

BACKGROUND

Insulin is a peptide hormone, produced by beta cells of the pancreas. Enzymatic cleavage of proinsulin results in the production of equimolar amounts of insulin and C-peptide in circulation. Insulin is central to regulating carbohydrate and fat metabolism in the body. Excessive amounts of insulin are associated with excess amounts of glucose in the system. High levels of insulin are known to cause weight gain, water bloating, high blood pressure, magnesium deficiency and an increase in the amount of inflammatory compounds in the blood, which causes blood clots and blood vessel damage. Insulin, when insufficiently produced, results in diabetes mellitus. In most cases, a high fasting insulin level is consistent with insulin resistance symptoms, but in some cases, it can be caused by more serious conditions such as Cushing's syndrome, acromegaly or possibly insulinoma.

PRINCIPLE

This ELISA kit is designed, developed and produced for the quantitative measurement of human Insulin in serum and /or EDTA-plasma samples. The assay utilizes the "sandwich" technique with selected antibodies that bind to various epitopes of Insulin. Assay standards, controls and patient samples are added directly to wells of a microplate that is coated with an anti-human Insulin specific antibody. Simultaneously, a horseradish peroxidase-conjugated monoclonal Insulin specific antibody is added to each well. After the first incubation period, the antibody on the wall of microtiter well captures human Insulin in the sample and unbound proteins in each microtiter well are washed away. A "sandwich" of "anti-Insulin antibody --- human Insulin --- HRP conjugated tracer antibody" is formed.

The unbound tracer antibody is removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to human Insulin on the wall of the microtiter well is directly proportional to the amount of Insulin in the sample. A standard curve is generated by plotting the absorbance versus the respective human Insulin concentration for each standard on point-to-point or 4 parameter curve fit. The concentration of human Insulin in test samples is determined directly from this standard curve.

ASSAY RESTRICTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

STORAGE CONDITIONS

The unopened kit should be stored at 2-8˚C for 1 year. Immediately after use remaining reagents should be returned to cold storage at 4˚C

MATERIALS SUPPLIED IN THIS KIT

1. Anti-human Insulin Antibody Coated Microplate: One microplate with 12 by 8 strips (96 wells total) coated with anti-human Insulin antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

2. Insulin Tracer Antibody: One vial containing 0.6 mL HRP-labeled Insulin antibody in a stabilized protein matrix. This reagent should be diluted with Insulin Tracer Antibody diluent and should be stored at 2 – 8°C. It is stable until the expiration date on the kit box.

3. ELISA Wash Concentrate: One bottle containing 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

4. ELISA HRP Substrate: One bottle containing 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. ELISA Stop Solution: One bottle containing 12 mL of stop solution. This reagent may be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

6. Human Insulin Standards: Five vials containing recombinant human Insulin in a

lyophilized bovine serum-based matrix with a non-azide preservative. Refer to the vials for exact concentration of the standard. These standards should be stored at 2 – 8°C and are stable until the expiration date on the kit box. Refer to assay procedure section for reconstitution instructions.

7. Human Insulin Controls: Two vials containing human Insulin in a lyophilized bovine serum based matrix with a non-azide preservative. Refer to vials for exact concentration range for each control. Both controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box. Refer to assay procedure section for reconstitution instructions.

8. Tracer Antibody Diluent: One bottle containing 12 mL ready-to-use buffer. It should be used only for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

PRECAUTIONS

Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8 °C in their pouch with the desiccant provided. Mix all reagents before using. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25 °C).

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use

separate reservoirs for each reagent.

• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

• When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

• Substrate solution should remain colourless until added to the plate. Keep Substrate solution protected from light. Substrate solution should change from colorless to gradations of blue.

• Stop Solution should be added to the plate in the same order as the Substrate solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate solution.

SAMPLE COLLECTION & STORAGE

Serum, EDTA-plasma, and urine samples are suitable specimens for human Insulin measurement. Only 50 µL of human sample is required for a duplicate determination of human Insulin with this test kit. No special preparation of the individual is necessary prior to specimen collection. Samples should be collected by standard technologies of the clinical laboratory practices and recommended by the manufacturer of sample collection tube. It is extremely important to carefully separate the serum and plasma from blood cells to avoid hemolyzation, etc. Serum/EDTA-plasma should be transferred to a clean test tube right after centrifugation. Human samples should be stored at 2 – 8ºC if the assay is to be performed within 72 hours. Otherwise, patient samples should be stored at –20ºC or below until measurement. Avoid more than three times freeze-thaw cycles of

specimen. Do not use hemolyzed, hyperlipermic, heat-treated or any contaminated specimens.

REAGENT PREPARATION

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate must be diluted to working solution prior to use.

(3) Reconstitute assay standards and controls by adding 0.5 mL of deminerialized water to each standard and control bottle. Allow the standards and controls to sit undisturbed for 5 minutes, then mix well by inversions or gentle vortexing. Make sure that all solid is dissolved completely prior to use. These reconstituted standards and controls may be stored at 2- 8°C for up to 3 days or below –20°C for long-term storage. Do not exceed 3 freeze-thaw cycles.

(4) Prepare Tracer Antibody working solution by 1:21 fold dilution of the Insulin Tracer Antibody by adding the tracer antibody into the Tracer Antibody Diluent . Following is a table that outlines the relationship of strips used and antibody mixture prepared. NOTE: the tracer antibody should be prepared just prior to the end of the first incubation cycle.

Dilution Scheme	Tracer Antibody Diluent	Tracer Antibody
1	1 mL	50 µL
2	2 mL	100 µL
3	3 mL	150 µL
4	4 mL	200 µL
5	5 mL	250 µL
6	6 mL	300 µL
7	7 mL	350 µL
8	8 mL	400 µL
9	9 mL	450 µL
10	10 mL	500 µL
11	11 mL	550 µL
12	12 mL	600 µL

(5) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3	STRIP 4
A	STD 1	STD 5	SAMPLE 1	SAMPLE 5
B	STD 1	STD 5	SAMPLE 1	SAMPLE 5
C	STD 2	STD 6	SAMPLE 2	SAMPLE 6
D	STD 2	STD 6	SAMPLE 2	SAMPLE 6
E	STD 3	C 1	SAMPLE 3	
F	STD 3	C 1	SAMPLE 3	
G	STD 4	C 2	SAMPLE 4	
H	STD 4	C 2	SAMPLE 4	

(6) Place a sufficient number of Anti-human Insulin antibody-coated microwell strips in a holder to run human Insulun.

NB.— Please note for conversion: 1 µg/L = 23 mU/L; 1 mU/L = 6.0 pmol/L

ASSAY PROCEDURE

(1) Add 25 µL of Standards, Controls and patient samples into the designated microwells.

(2) Add 100 µL of the above diluted Tracer Antibody working solution to each well.

(3) Seal the plate wells securely, cover with foil or similar material to protect from light. Incubate the plate shaking, at room temperature for 1 hr. ± 5 minutes.

(4) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(5) Add 100 µL of ELISA HRP Substrate into each of the wells.

(6) Cover the plate with aluminum foil or similar material to avoid exposure to light. Incubate the plate static, at room temperature for 20 minutes.

(7) Immediately add 100 µL of ELISA Stop Solution into each of the wells. Mix

gently.

(8) Read the absorbance at 450 nm with reference filter at 620 nm or 650 nm.

EXPECTED VALUES AND CHARACTERISTICS

Human non-fasting samples from normal healthy adults ages 20 – 60 were collected and measured with this ELISA. The recommended normal high cut-off for Insulin concentration by using this ELISA is 3 ng/mL with an average level of 0.652 ng/mL (range 0.12 – 2.7 ng/mL, SD 0.701 ng/mL). We strongly recommend for each laboratory to establish its own normal range by measuring EDTA plasma and/or serum with this ELISA.

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical sensitivity (LLOD) of the Insulin ELISA as determined by the 95% confidence limit on 16 duplicate determination of zero standard is approximately 0.1817 mU/L.

High Dose “hook” effect

This assay has showed that it did not have any high dose “hook” for Insulin levels up to 4600 mU/L.

Specificity

This assay measures human Insulin without any cross-reaction to C-peptide.

Linearity was checked by assaying dilution series of 5 samples with different Insulin concentrations. Linearity percentages obtained ranged within 90 to 110%.

Recovery was estimated by assaying 5 mixed samples with known Insulin concentrations. The recovery percentages ranged from 85 to 105%

CALCULATION

It is recommended to use a point-to-point or 4-parameter standard curve fitting.

1. Calculate the average absorbance for each pair of duplicate test results.

2. Subtract the average absorbance of the level 1 standard (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.

3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results. The human Insulin concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance.

VALIDITY & STORAGE: 12 months (at 2-8℃, unopened).