Epitope Diagnostics, Inc.

EDI™ Human Ultra Sensitive Insulin ELISA

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Quantitative Measurement of Human Insulin Levels in blood

REF **KT-887** EU: IVD CE Σ 96 +2 +8 °C

INTENDED USE
This test kit is intended for use in the quantitative determination of low level of human Insulin in serum or plasma.

SUMMARY OF PHYSIOLOGY
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.

ASSAY 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.

REAGENTS: Preparation and Storage
This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use, allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

1. **Anti-human Insulin Antibody Coated Microplate (Cat. No. 30740)**
One microplate with twelve by eight 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 (Cat. No. 30751)**
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 (Cat. No. 10010)**
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 (Cat. No. 10020)**
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 (Cat. No. 10030)**
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 (Cat. No. 30781 – 30786)**
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 (Cat. No. 30787 – 30788)**
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 (Cat. No. 30710)
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.

SAFETY PRECAUTIONS

The reagents must be used in professional laboratory. Source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 100 µL.
2. Disposable pipette tips suitable for above volume dispensing.
3. Aluminum foil.
4. Deionized or distilled water.
5. Plastic microtiter well cover or polyethylene film.
6. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
7. Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm.

SPECIMEN COLLECTION

Serum and EDTA-plasma are suitable specimens for human Insulin measurement. Only **100 µL** of human sample is required for a duplicate determination of low human Insulin with this test kit. No special preparation of individual is necessary prior to specimen collection. Samples should be collected by standard technologies of clinical laboratory practices and recommended by the manufacturer of the 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.

ASSAY PROCEDURE

1. 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 (Cat. 10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.

(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 (Cat. 30751) by adding the tracer antibody into the Tracer Antibody Diluent (Cat. 30710). 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	0.5 mL	25 µL
2	1 mL	50 µL
3	1.5 mL	75 µL
4	2 mL	100 µL
5	2.5 mL	125 µL
6	3 mL	150 µL
7	3.5 mL	175 µL
8	4 mL	200 µL
9	4.5 mL	225 µL
10	5 mL	250 µL
11	5.5 mL	275 µL
12	6 mL	300 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 (Cat. 30740) in a holder to run human Insulun standards, controls and unknown samples in duplicates.

2. Assay Procedure:

(1) Add **50 µL** of Standards, Controls and patient samples into the designated microwells.

(2) Add **50 µL** of the above diluted Tracer Antibody working soution 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 **2 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 (Cat. 10020) 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 (Cat. 10030) into each of the wells. Mix gently.
(8) Read the absorbance at 450 nm with reference filter at 620 nm or 650 nm.

PROCEDURAL NOTES

1. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
8. If adapting this assay to automated ELISA system such as DS-2 (Diamedix Corp., Miami), a procedural validation is necessary if there is any modification of the assay procedure.

INTERPRETATION OF RESULTS

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

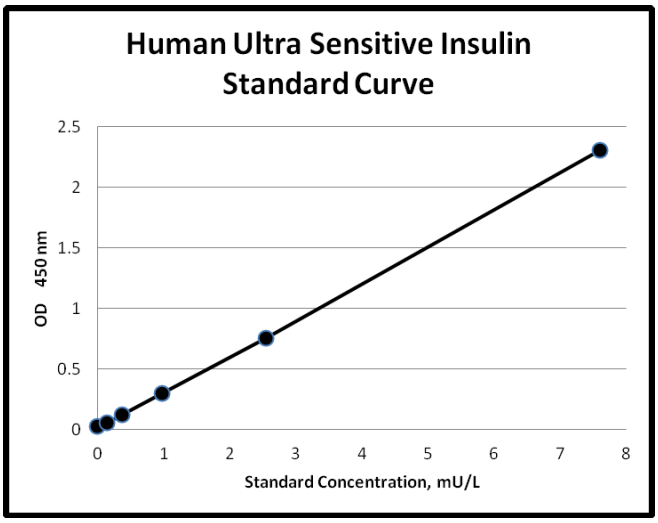
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the level 1 standard (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard 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.

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from this Human Insulin ELISA are represented. **This curve should not be used in lieu of standard curve generated with each assay.**

Well I.D.	OD 450/620 nm Absorbance			Results mU/L
	Readings	Average	Corrected	
Std-1: 0 mU/L	0.031 0.026	0.029	0	
Std-2: 0.145 mU/L	0.060 0.063	0.061	0.032	
Std-3: 0.377 mU/L	0.119 0.127	0.123	0.940	
Std-4: 0.982 mU/L	0.328 0.271	0.299	0.270	
Std-5: 2.553 mU/L	0.770 0.740	0.755	0.726	
Std-6: 7.60 mU/L	2.209 2.399	2.304	2.275	
Control 1	0.189 0.194	0.192	0.163	0.612
Control 2	0.486 0.540	0.498	0.469	1.668



EXPECTED VALUES

Human non-fasting and 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 65 mU/L with an average level of 15 mU/L (range 2.76 – 62.1 mU/mL, SD 16 mU/L). We strongly recommend for each clinical laboratory to establish its own normal range by measuring EDTA plasma and/or serum with this ELISA.

LIMITATION OF THE PROCEDURE

1. An abnormally high Insulin test result cannot be independently used for clinical diagnosis. As with other laboratory tests, a variety of analytical and pre-analytical factors may lead to false high test results. Physicians must interpret the test result in the light of each patient's clinical findings.
2. For sample values reading greater than the highest standard, it is recommended to re-assay samples with further dilutions (i.e. 1:10 or 1:100 with 5%BSA in 0.01M PBS).
3. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls.

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity (LLOD) of the Insulin ELISA as determined by the 95% confidence limit on 16 duplicate determination of zero standard is approximately 0.0322 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.

Precision
The intra-assay precision was validated by measuring three control samples with 16 replicate determinations.

Sample #	Mean Insulin Value (mU/L)	CV (%)
1	2.98	8.4
2	2.04	6.3
3	3.29	9.2

The inter-assay precision was validated by measuring two control levels in duplicate in 6 individual assays.

Sample #	Mean Insulin Value (mU/L)	CV (%)
1	0.70	8.3
2	1.89	9.9

Linearity
Three samples were collected and tested. The results of Insulin percent recovery value in mU/L are as follows:

DILUTION	OBSERVED VALUE (mU/L)	RECOVERY
Neat A	5.89	-
1:2	2.97	100.8
1:4	1.66	112.7
1:8	0.92	125.0
Neat B	3.06	-
1:2	1.57	102.6
1:4	0.86	112.4
1:8	0.36	94.1
Neat C	0.98	-
1:2	0.52	106.1
1:4	0.24	98.0
1:8	0.13	108.4

Spike Recovery
Three samples and three assay standards (0.377, 0.982 and 2.553 mU/L) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (mU/L)	RECOVERY
Neat A	3.32	-
Std-3: 0.377 mU/L	2.17	117.4
Std-4: 0.982 mU/L	2.52	117.2
Std-5: 2.553 mU/L	3.37	114.8
Neat B	3.02	-
Std-3: 0.377 mU/L	1.61	94.8
Std-4: 0.982 mU/L	1.90	95.0
Std-5: 2.553 mU/L	2.74	98.3
Neat C	5.89	-
Std-3: 0.377 mU/L	3.44	109.8
Std-4: 0.982 mU/L	3.55	103.3
Std-5: 2.553 mU/L	4.10	97.1

CONVERSION FACTOR
1 µg/L = 23 mU/L; 1 mU/L = 6.0 pmol/L

WARRANTY
This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCE

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2) Rudovich NN, Rochlitz HJ, Pfeiffer AF. (2004) Reduced hepatic insulin extraction in response to gastric inhibitory polypeptide compensates for reduced insulin secretion in normal-weight and normal glucose tolerant first-degree relatives of type 2 diabetic patients. *Diabetes* 53:2359-2365
3) Lindstrom T, Hedman CA, Arnquist HJ. (2002). Use of a novel double-antibody technique to describe the pharmacokinetics of rapid-acting insulin analogs *Diabetes Care* 25:1049-105454
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5) Chevenne D., Ruiz J., Lohmann L., et.al.: Immunoradiometric Assay of Human Intact Proinsulin Applied to Patients with Type 2 Diabetes, Impaired Glucose Tolerance, and Hyperandrogenism. Clinical Chemistry. 40/5:754, 1994

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.
www.epitopediagnostics.com

This product is developed and manufactured by

Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, USA

MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
In Vitro Diagnostic Device	Use by
Read instructions before use	Lot No.
Authorized Representative In Europe	

Ultra Sensitive Insulin ELISA: Condensed Assay Protocol

1. **50 µL Standards, controls, and patient samples**

↓ *Immediately*

2. **50 µL Tracer Antibody**

↓ *Incubate @ RT for 2 hours shaking*
Wash 5 x

3. **100 µL TMB Substrate**

↓ *Incubate @ RT for 20 min. static*

4. **100 µL Stop Solution**

↓ *Immediately*

5. **Read absorbance at 450/650 or 450/620 nm** *within 10 minutes*