EPITOPE DIAGNOSTICS, INC.

EDI™ Human Insulin ELISA

Enzyme Linked Immunosorbent Assay (ELISA) for the Quantitative Measurement of Human Insulin Levels in serum or plasma

REF KT-886 CE IVD 96

INTENDED USE

This test kit is intended for use in the quantitative determination of human Insulin in serum or plasma. This kit is for in vitro diagnostic use only.

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 calibrators, controls and patient samples are added directly to wells of a microplate that is coated with Insulin monoclonal antibody. Simultaneously, a horseradish peroxidase-conjugated Insulinspecific 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 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 calibration curve is generated by plotting the absorbance versus the respective human Insulin concentration for each calibrator on point-to-point or 4 parameter curve fit. The concentration of human Insulin in test samples is determined directly from this calibration curve.

The EDI™ Human Insulin ELISA has been calibrated against WHO International Standard 83/500.

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. Human Insulin Coated Microplate (30740)

Microplate coated with Insulin monoclonal antibody.

Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use.

2. Insulin Tracer Antibody (30751)

HRP-labeled insulin antibody in a stabilized protein matrix.

Qty: 1 x 12 mL.
Storage: 2 – 8°C
Preparation: Ready to Use.

3. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty: 1 x 30 mL
Storage: 2 – 25°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL demineralized water and mixed well before use.

4. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

5. ELISA Stop Solution (10030)

0.5 M sulfuric acid

Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to Use.

6. Human Insulin Calibrators Levels 1- 6 (30771 - 30776)

Recombinant human Insulin in a lyophilized bovine serum-based matrix with a non-azide preservative. Refer to the vials for exact concentration.

Qty: 6 x Vials
Storage: 2 – 8°C (Lyophilized), <-20°C (Reconstituted)
Do not exceed 3 freeze-thaw cycles.
Preparation: Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

7. Human Insulin Controls (30777, 30778)

Recombinant human Insulin in a lyophilized bovine serum-based matrix with a non-azide preservative. Refer to the vials for exact concentration.

Qty: 2 x Vials

Storage: 2 – 8°C (Lyophilized), <-20°C (Reconstituted)
Do not exceed 3 freeze-thaw cycles.

Preparation: Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic use only. Source material which contains reagents of bovine serum albumin 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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 & 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.

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 (10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.
3. Reconstitute assay calibrators and controls by adding 0.5 mL of demineralized water to each calibrator and control bottle. Allow the calibrators 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 calibrators 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.

2. Manual Assay Procedure

1. Place a sufficient number of microwell strips (30740) in a holder to run calibrators (30771 - 30776), controls (3077, 30778), and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 1
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 1
C	Calibrator Level 2	Calibrator Level 6	SAMPLE 2
D	Calibrator Level 2	Calibrator Level 6	SAMPLE 2
E	Calibrator Level 3	Control 1	SAMPLE 3
F	Calibrator Level 3	Control 1	SAMPLE 3
G	Calibrator Level 4	Control 2	SAMPLE 4
H	Calibrator Level 4	Control 2	SAMPLE 4

3. Add **25 µL** of calibrators (30771 - 30776), controls (3077, 30778), and samples into the designated microwells.
4. Add **100 µL** of tracer antibody (30751) into each microwell.
5. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** with **shaking at 350 to 450 rpm** for **60 minutes.**
6. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
7. Add **100 µL** of substrate (10020) into each microwell.
8. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
9. Add **100 µL** of stop solution (10030) into each microwell, Mix by gently tapping the plate.
10. Read the absorbance at **450/620 nm** or **450/650 nm** within **10 minutes** with a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all calibrators, 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.

INTERPRETION OF RESULTS

1. It is recommended to use a 4-parameter calibration curve fitting.
2. Calculate the average absorbance for each pair of duplicate test results.
3. Subtract the average absorbance of the level 1 calibrator (0 mIU/L) from the average absorbance of all other readings to obtain corrected absorbance.
4. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using 4-parameter curve. Appropriate computer assisted data reduction programs may also be used for the calculation of results.
5. The human Insulin concentrations for the controls and the patient samples are read directly from the calibration curve using their respective corrected absorbance.

LIMITATIONS 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 calibrator, 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.

The EDI™ Human Insulin ELISA has been calibrated against WHO International Standard 83/500.

EXPECTED VALUES

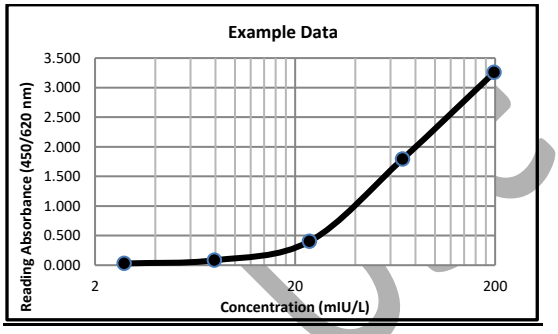
Human non-fasting and fasting samples from normal healthy adults ages 20 – 60 were collected and measured with this ELISA. The recommended normal high cut-off for Insulin concentration by using this ELISA is 102.1mIU/L with an average level of 23.6mIU/L (range 4.33 – 97.5mIU/L). We strongly recommend for each clinical laboratory to establish its own normal ranges of fasting and non-fasting population by measuring EDTA plasma and/or serum with this ELISA.

EXAMPLE DATA

A typical absorbance data and the resulting calibration curve from are represented.

Note: This curve should not be used in lieu of calibration curve run with each assay.

Well ID	Reading Absorbance (450/620 nm)			Concentration (mIU/L)
	Readings	Average	Corrected	
Calibrator Level 1: 0 mIU/L	0.004	0.004	0.000	
	0.004			
Calibrator Level 2: 2.8 mIU/L	0.029	0.029	0.025	
	0.029			
Calibrator Level 3: 7.9 mIU/L	0.087	0.083	0.079	
	0.079			
Calibrator Level 4: 23.6 mIU/L	0.381	0.403	0.399	
	0.424			
Calibrator Level 5: 69 mIU/L	1.792	1.796	1.792	
	1.800			
Calibrator Level 6: 198 mIU/L	3.247	3.258	3.254	
	3.268			
Control 1	0.185	0.204	0.200	15.5
	0.223			
Control 2	0.863	0.863	0.859	38.1
	0.862			



PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity (LLOD) of the Insulin ELISA as determined by the 95% confidence limit on 16 duplicate determination of zero calibrator is approximately 0.1928mIU/L. The LOQ of this test is 0.8789 mIU/L.

Hook Effect

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

Specificity

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

Reproducibility and Precision

The intra-assay precision was validated by measuring three control samples with 16 replicate determinations. The inter-assay precision was validated by measuring two control levels in duplicate in 9 individual assays. The results are as follows:

	Inter-Assay		Intra-Assay		
Sample	1	2	1	2	3
Mean (mIU/L)	16.56	51.29	11.20	44.11	20.2
CV (%)	9.4	8.0	7.8	3.1	9.0

Linearity

Three samples were collected and tested. The results are as follows:

Samples	Observed (mIU/L)	Expected (mIU/L)	Recovery (%)
Sample A	198.0	-	-
50%	99.0	99.4	100
25%	49.5	46.9	95
12.5%	24.8	23.9	97
Sample B	69.0	-	-
50%	34.5	28.7	83
25%	17.25	17.6	102
12.5%	8.6	9.4	109
Sample C	23.6	-	-
50%	11.8	12.1	103

25%	5.9	6.7	113
12.5%	2.9	2.9	97

Spike Recovery
Three samples and three assay calibrators (7.60, 23 and 69 mIU/L) were combined at equal volumes and tested. The results indicate below:

Samples	Observed (mIU/L)	Recovery (%)
Sample A	17.80	-
+ Level 3	12.12	95.4
+ Level 4	17.14	84.0
+ Level 5	37.56	86.5
Sample B	30.12	-
+ Level 3	16.01	84.9
+ Level 4	22.43	84.5
+ Level 5	51.12	103.0
Sample C	10.12	-
+ Level 3	7.36	83.1
+ Level 4	14.05	84.8
+ Level 5	39.03	98.7

WARRANTY
This product is warranted to perform as described in its labeling and literature when used in accordance c 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.

REFERENCES

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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.

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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

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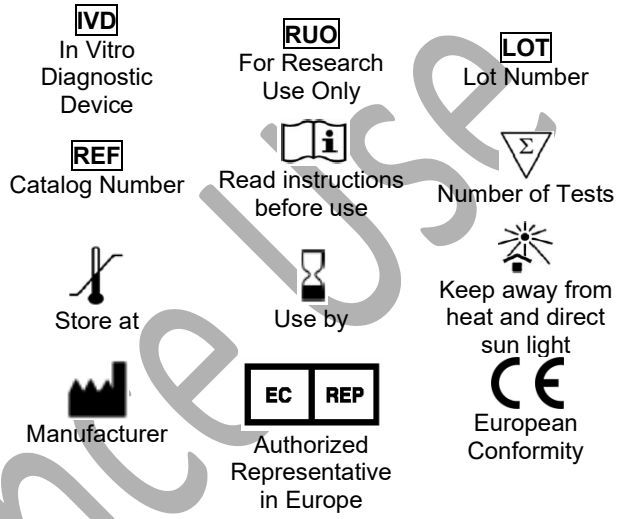
GLOSSARY OF SYMBOLS (EN 980/ISO 15223)



SHORT ASSAY PROCEDURE
1. Add **25 µL** of Calibrators, Controls and patient samples into the designated microwells.
2. Add **100 µL** of Tracer Antibody to each microwell.
3. Cover and incubate at **room temperature (20-25 ºC)** with **shaking at 350 to 450 rpm** for **60 minutes.**
4. Wash each well five times.
5. Add **100 µL** of substrate to each well.
6. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes.**
7. Add **100 µL** of stop solution to each well.
8. Read the absorbance at **450/620 nm** or **450/650 nm.**