Product information





Users Manual

Insulin ELISA

Enzyme immunoassay for the measurement of Insulin in serum and plasma.



IB79167



96 wells



For in-vitro diagnostic use only.

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1 INTRODUCTION

1.1 Intended Use

The **IBL-AMERICA Insulin ELISA** is an enzyme immunoassay for the *in vitro diagnostic* measurement of Insulin in serum and plasma (heparin- or EDTA plasma.

1.2 Summary and Explanation

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 C-peptide 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.

2 PRINCIPLE OF THE TEST

The IBL-AMERICA Insulin ELISA kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on the Insulin molecule. An aliquot of unknown containing endogenous Insulin is incubated in the coated well with enzyme conjugate, which is an anti-Insulin antibody conjugated with Biotin. After incubation the unbound conjugate is washed off. During the second incubation step Streptavidin Peroxidase Enzyme Complex binds to the biotin-anti-Insulin antibody. The amount of bound HRP complex is proportional to the concentration of Insulin in the unknown. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Insulin in the unknown.

3 WARNINGS AND PRECAUTIONS

- 1. This kit is for in vitro diagnostic use only. For professional use only.
- 2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- 3. Before starting the assay, read the instructions completely and carefully. <u>Use the valid version of the package insert provided with the kit</u>. Be sure that everything is understood.
- 4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
- 5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- 6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution coloured. Do not pour reagents back into vials as reagent contamination may occur.
- 7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- 8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- 9. Allow the reagents to reach room temperature (21 °C 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
- 10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- 11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- 12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- 13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- 14. Do not use reagents beyond expiry date as shown on the kit labels.
- 15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- 16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- 17. Avoid contact with *Stop Solution* containing 1 N acidic solution. It may cause skin irritation and burns.
- 18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
- 19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- 20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- 21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from IBL-AMERICA.

4 REAGENTS

4.1 Reagents provided

- 1. **SORB MT Microtiterwells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-Insulin antibody (monoclonal).
- 2. **CAL Q** Zero Standard, 1 vial, 3 mL, ready to use. 0 µIU/mL. Contains non-mercury preservative.
- B. **CAL** 1 5 Standard (Standard 1-5), 5 vials, 1 mL, ready to use;

Concentrations: 6.25 - 12.5 - 25 - 50 and 100 µIU/mL,

Conversion: $\mu IU/mL \times 0.0433 = ng/mL$,

ng/mL x 23.09 = $\mu IU/mL$

The standards are calibrated against international WHO approved Reference material NIBSC 66/304.:

Contain non-mercury preservative.

- 4. **ENZ CONJ Enzyme Conjugate**, 1 vial, 5 mL, ready to use, mouse monoclonal anti-Insulin conjugated to biotin; Contains non-mercury preservative.
- 5. **ENZ COMP Enzyme Complex**, 1 vial, 7 mL, ready to use, Streptavidin-HRP Complex. Contains non-mercury preservative.
- 6. **SUB TMB Substrate Solution**, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
- 7. **STOP SOLN Stop Solution**, 1 vial, 14 mL, ready to use, contains 0.5 M H₂SO₄. Avoid contact with the stop solution. It may cause skin irritations and burns.
- WASH SOLN 40x Wash Solution, 1 vial, 30 mL (40X concentrated), see "Preparation of Reagents".

Note: Additional Zero *Standard* for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionised water
- Timer
- Graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 $^{\circ}$ C to 8 $^{\circ}$ C. Microtiter wells must be stored at 2 $^{\circ}$ C to 8 $^{\circ}$ C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for 3 months if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.

Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL. *The diluted Wash Solution is stable for 2 weeks at room temperature.*

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, IBL-AMERICA has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 COLLECTION AND HANDLING OF UNKNOWNS

Serum or plasma (only heparin- or citrate plasma) can be used in this assay. Please note: Samples containing sodium azide should not be used in the assay. In general, it should be avoided to use haemolytic, icteric or lipaemic specimens. For further information refer to chapter "Interfering Substances".

5.1 Collection of Unknowns

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Storage and Preparation of Unknowns

Unknowns should be capped and may be stored for up to 7 days at 2 °C to 8 °C prior to assaying. Unknowns held for a longer time (up to 18 months) should be frozen only once at -20 °C prior to assay. Thawed unknowns should be inverted several times prior to testing.

5.3 Dilution of Unknowns

If in an initial assay, an unknown is found to contain more than the highest standard, the unknowns can be diluted with *Zero Standard* and re-assayed as described in Assay Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

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

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and unknowns must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or unknown in order to avoid cross contamination.
- 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.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

- Secure the desired number of Microtiter wells in the frame holder.
- 2. Dispense **25 μL** of each **Standard, control** and **unknowns** <u>with new disposable tips</u> into appropriate wells.
- 3. Dispense **25 µL** *Enzyme Conjugate* into each well.

 Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
- 4. Incubate for **30 minutes** at room temperature.
- 5. Briskly shake out the contents of the wells.

Rinse the wells 3 times with 400 μL diluted Wash Solution per well, if a plate washer is used

- or –

Rinse the wells **3 times** with **300 \muL** diluted Wash Solution per well for manual washing. Strike the wells sharply on absorbent paper to remove residual droplets.

Important note:

The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

- 6. Add **50 μL** of *Enzyme Complex* to each well.
- 7. Incubate for **30 minutes** at room temperature.
- 8. Briskly shake out the contents of the wells.

Rinse the wells 3 times with 400 μ L diluted Wash Solution per well, if a plate washer is used - or –

Rinse the wells **3 times** with **300 \muL** diluted Wash Solution per well for manual washing. Strike the wells sharply on absorbent paper to remove residual droplets.

- 9. Add **50 μL** of **Substrate Solution** to each well.
- 10. Incubate for **15 minutes** at room temperature.
- 11. Stop the enzymatic reaction by adding 50 µL of Stop Solution to each well.
- 12. Determine the absorbance (OD) of each well at **450 nm** (reading) and at **620-630 nm** (background subtraction, recommended) with a microtiter plate reader. It is recommended that the wells be read within **10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

- 1. Calculate the average absorbance values for each set of standards, controls and unknowns.
- 2. Using linear graph paper, construct a calibration 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 unknown determine the corresponding concentration from the calibration curve.
- 4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.)
 - Other data reduction functions may give slightly different results.
- 5. The concentration of the unknowns can be read directly from this calibration curve. Unknowns 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.

6.3.1 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.

Standard	Optical Units (450 nm)
Standard 0 (0 µIU/mL)	0.03
Standard 1 (6.25 µIU/mL)	0.07
Standard 2 (12.5 µIU/mL)	0.14
Standard 3 (25 µIU/mL)	0.35
Standard 4 (50 µIU/mL)	0.88
Standard 5 (100 µIU/mL)	2.05

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 IBL-America Insulin ELISA the following values are observed:

2 uIU/mL to 25 uIU/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 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IBL-AMERICA directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between $1.76-100\ \mu IU/mL$.

9.2 Specificity of Antibodies (Cross Reactivity)

The cross reactivities were determined by addition of different analytes to serum containing 4 ng/mL (≅ 100 µIU/mL) Insulin and measuring the apparent Insulin concentration.

Added analyte to a hi	_	Observed Insulin value (ng/mL)	Cross reaction (%)
Porcine Insulin	8 ng/mL	17	> 100
Bovine Insulin	8 ng/mL	17.8	> 100
Dog Insulin	16 ng/mL	17.2	82
Rabbit Insulin	16 ng/mL	14.1	63
Rat Insulin	16 ng/mL	4.0	0
Human Proinsulin	32 ng/mL	4.1	0
Porcine Proinsulin	16 ng/mL	4.0	0
Bovine Proinsulin	16 ng/mL	4.1	0

9.3 Sensitivity

The <u>analytical sensitivity</u> of the IBL-AMERICA ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Zero Standard and was found to be 1.76 µIU/mL.

9.4 Reproducibility

9.4.1 Intra-Assay

The within assay variability is shown below:

Sample	n	Mean (µIU/mL)	CV (%)
1	20	17.5	2.6
2	20	66.4	1.8

9.4.2 Inter-Assay

The between assay variability is shown below:

no botti oon acca		y variability to ofform botom.		
Sample	n	Mean (µIU/mL)	CV (%)	
1	12	17.4	2.9	
2	12	66.9	6.0	

9.5 Recovery

Samples have been spiked by adding Insulin solutions with known concentrations in a 1:1 ratio. The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

		Sample 1	Sample 2
Concentration (µIU/mL)		21.2	69.0
Average Recovery (%)		106.5	91.8
Range of Recovery (%)	from	101.1	91.8
Kange of Recovery (76)	to	109.6	100.1

9.6 Linearity

Samples were measured undiluted and in serial dilutions with zero standard. The recovery (%) was calculated by multiplying the ratio of expected and measured values with 100.

		Sample 1	Sample 2
Concentration (µIU/mL)		21.2	69.0
Average Recovery (%)		100.8	100.5
Range of Recovery (%)	from	88.5	88.4
Range of Recovery (70)	to	110.3	110.4

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), bilirubin (up to 0.5 mg/mL) and triglyceride (up to 30 mg/mL) have no influence on the assay results.

A biotin concentration of up to 1200 ng/mL in a sample has no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Insulin in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 1600 µIU/mL of Insulin.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IBL-AMERICA.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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12 REFERENCES / LITERATURE

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SYMBOLS USED WITH IBL-AMERICA ELISAS

Symbol	English	Deutsch	Français	Español	Italiano
((European Conformity	CE-Konformitäts- kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
[]i	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	Contains sufficient for <n> tests/</n>	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos</n>	Contenuto sufficiente per "n" saggi
1	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
\square	Expiration Date	Mindesthaltbarkeits- datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
***	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Contenu	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
Microtiterwells	Microtiterwells	Mikrotiterwells	Plaques de micro- titration	Placas multipocillo	Micropozzetti
Antiserum	Antiserum	Antiserum	Antisérum	Antisuero	Antisiero
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Enzyme Complex	Enzyme Complex	Enzymkomplex	Complexe enzymatique	Complex enzimático	Complesso enzimatico
Substrate Solution	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
Stop Solution	Stop Solution	Stopplösung	Solution d'arrêt	Solución de parada	Soluzione d' arresto
Zero Standard	Zero Standard	Nullstandard	Zero Standard	Estándar cero	Standard zero
Standard	Standard	Standard	Standard	Estándar	Standard
Control	Control	Kontrolle	Contrôle	Control	Controllo
Assay Buffer	Assay Buffer	Assaypuffer	Tampon d'essai	Tampón de ensayo	Tampone del test
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH (idrossido di sodio 1N)
1 N HCI	1 N HCI	1 N HCI	1N HCI	1 N HCI	
Sample Diluent	Sample Diluent	Probenverdünnungs- medium	Solution pour dilution de l'échantillon	Solución para dilución de la muestra	Diluente dei campioni
Conjugate Diluent	Conjugate Diluent	Konjugatverdünnungs- medium	Solution pour dilution du conjugué	Solución para dilución del conjugado	Diluente del tracciante