AccuDiag™ Insulin ELISA Kit

REF 1606-15

IVD See External Label 2°C 8°C Σ 96 Tests

Insulin ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0.7-9.0 µIU/ml
Sample	50 µL
Specificity	>99%
Sensitivity	0.182 µIU/ml
Incubation Time	135 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

Diagnostic Automation, Inc. Insulin Microplate Elisa test is intended to be used for the quantitative determination of insulin levels in human serum.

SUMMARY AND EXPLANATION

Human insulin is a peptide produced in the beta cells of the pancreas and is responsible for the metabolism and storage of carbohydrates. As a result of biofeedback the insulin levels increase with intake of sugars and decline when sugar content is low for absorption. In the diabetic population the mechanism of insulin production is impaired because of genetic predispositions (**Type I**) or because of lifestyle and/or hereditary factors (**Type II**). In such cases either the insulin production has to be boosted by medication or it has to be supplemented by oral or intravenous methods. The quantitative determination of insulin can help in dose selection the patient has to be subjected to.

On the other hand the circulatory insulin can be found at much higher levels in patients with pancreatic tumors. These tumors secrete abnormally high levels of insulin and thus cause hypoglycemia. Accordingly, fasting hypoglycemia associated with inappropriately high concentrations of insulin strongly suggests an islet-cell tumor (insulinoma). To distinguish insulinomas from factitious hypoglycemia due to insulin administration, serum C-peptide values are recommended. These insulinomas can be localized by provocative intravenous doses of *tolbutamide* and *calcium*.

ASSAY PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (Ab), (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess**, and native antigen (Ag). In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal insulin antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(M)} + Ag_{Ins} + {}^{Btn}Ab_{(M)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(M)}\text{–}Ag_{Ins}\text{–}{}^{Btn}Ab_{(M)}$$

$^{Btn}Ab_{(M)}$ = Biotinylated Monoclonal Ab (Excess Quantity)
Ag_{Ins} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(M)}$ = Enzyme labeled Monoclonal Ab (Excess Quantity)
$^{Enz}Ab_{(M)} - Ag_{Ins} - {}^{Btn}Ab(M)$ = Antigen-Antibodies complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$$^{Enz}Ab_{(M)} - Ag_{Ins} - {}^{Btn}Ab_{(M)} + \underline{Streptavidin}_{C.W.} \Rightarrow \underline{Immobilize\ complex}$$

$\underline{Streptavidin}_{C.W.}$ = Streptavidin immobilized on well
$\underline{Immobilized\ complex}$ = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS

Materials provided with the test kit

A. **Insulin Calibrators – 2.0 ml/vial (Dried) – [Icons A–F]** Six (6) vials of references for Insulin antigen at levels of 0(**A**), 5 (**B**), 25(**C**), 50(**D**), 100(**E**) and 300(**F**) µIU/ml. Reconstitute each vial with 2ml of distilled or deionized water. The reconstituted calibrators are stable for three (3) days at 2-8°C.

In order to store for a longer period of time, aliquot the reconstituted calibrators stored at -20°C for up to 30 days. **Do not freeze thaw more than once**. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRP 66/304.

B. **Insulin Enzyme Reagent —13ml/vial**
One (1) vial containing enzyme labeled affinity purified monoclonal mouse x-insulin IgG, biotinylated monoclonal mouse x-insulin IgG in buffer, dye, and preservative. Store at 2-8°C.
C. **Streptavidin Coated Plate – 96 wells**
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
D. **Wash Solution Concentrate – 20 ml**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
E. **Substrate A –7.0ml/vial**
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.
F. **Substrate B –7.0ml/vial**
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.
G. **Stop Solution – 8.0ml/vial**
One (1) bottle containing a strong acid (1N HCl). Store at 2-8°C.
H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.
Note 3: Above reagents are for a single 96-well microplate.

Materials required but not provided

1. Pipette(s) capable of delivering 0.050 ml (50µl) and 0.100ml (100 µl) volumes with a precision of better than 1.5%
2. Dispenser(s) for repetitive deliveries of 0.100ml (100 µl) and 0.350ml (350 µl) volumes with a precision of better than 1.5% (optional).
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader at 450 nm and 620 nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Storage container for storage of wash buffer.
10. Distilled or deionized water.
11. Quality Control Materials.

PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals
All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1968, HHS.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood: serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.
Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the samples(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution – Stable for one year.**
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).
****Test procedure should be performed by a skilled individual or trained professional.**

1. Format the microplates' wells for calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of the Insulin Enzyme Reagent to each well**. It is very**

important to dispense all reagents close to the bottom of the microwell.

4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap.
5. Incubate for 120 minutes at room temperature (20-27°C).
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Do not shake the plate after substrate addition**
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

RESULTS

A dose response curve is used to ascertain the concentration of Insulin in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Insulin concentration in µIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of Insulin for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µIU/ml) from the horizontal axis of the graph (the duplicate of the unknown may be averaged as indicated). In the following example, the average absorbance 0.624 intersects the dose response curve at 66.8 µIU/ml Insulin concentration (See Figure 1).

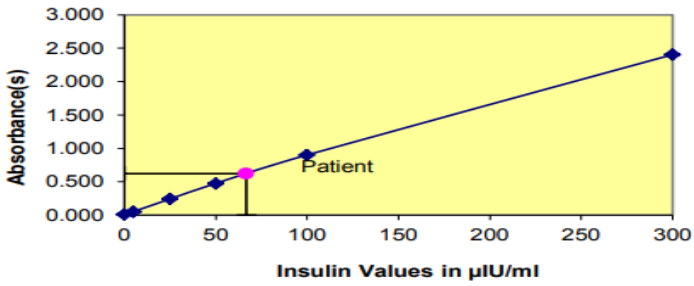
Note: Computer data reduction software designed for IEMA (ELISA) assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (µIU/ml)
Cal A	A1	0.011	0.010	0
	B1	0.009		
Cal B	C1	0.054	0.054	5
	D1	0.053		
Cal C	E1	0.244	0.243	25
	F1	0.241		
Cal D	G1	0.464	0.476	50
	H1	0.488		
Cal E	A2	0.882	0.902	100
	B2	0.922		
Cal F	C2	2.467	2.405	300
	D2	2.342		
Ctrl 1	E2	0.065	0.065	6.4
	F2	0.067		
Ctrl 2	G2	1.581	1.587	188.0
	H2	1.593		
Patient 1	A3	0.597	0.624	66.8
	B3	0.651		

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 µIU/ml should be ≤ 0.04.
2. The absorbance (OD) of calibrator 300 µIU/ml should be ≥ 1.8.
3. Four out of six quality control pools should be within the established ranges.

ASSAY PERFORMANCE

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimens(s) should not be used.
4. If more than once (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from

different batches.

9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from DAI IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

INTERPRETATION

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, DAI shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Patient samples with Insulin concentrations above 300µIU/ml may be diluted with the zero calibrator and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value. **An Insulin value alone is not of diagnostic value and should and should only be used in conjunction with clinical manifestations and diagnostic procedures.**

EXPECTED VALUES

Insulin values are consistently higher in plasma than in serum; thus, serum is preferred. Compared with fasting values in non-obese non-diabetic individuals, insulin levels are higher in obese non-diabetic subjects and lower in trained athletes. Although proinsulin cross reacts with most competitive insulin assays, there is less than 1% cross reaction found with proinsulin using DAI Insulin Microwell Elisa.

Based on the clinical data gathered by DAI in concordance with the published literature the following ranges have been assigned. **These ranges should be used as guidelines only:**

POPULATION	RANGE
Children < 12 yrs	< 10 µIU/ml
Adult (Normal)	0.7 – 9.0 µIU/ml
Diabetic (Type II)	0.7 – 25 µIU/ml

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

A. **Precision**

The within and between assay precisions of the DAI Insulin ELISA Microplate Test System were determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Tables 2 and 3.

TABLE 2
Within Assay Precision (Values in µIU/ml)

Sample	N	X	σ	C.V.
Pool 1	24	10.70	0.89	8.3%
Pool 2	24	48.16	2.07	4.3%
Pool 3	24	130.08	6.64	5.1%

TABLE 3
Between Assay Precision (Values in µIU/ml)

Sample	N	X	σ	C.V.
Pool 1	15	11.78	1.33	11.3%
Pool 2	15	48.92	4.69	9.6%
Pool 3	15	145.17	10.45	7.2%

B. **Sensitivity**

The sensitivity (detection limit) was ascertained by determining the variability of the 0 µIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.182 µIU/ml.

C. **Accuracy**

The DAI Insulin ELISA was compared with a reference coated tube radioimmunoassay assay. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from 0.01µIU/ml – 129 µIU/ml). The total number of such specimens was 104. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Lease Square Regression Analysis	Correlation Coefficient
This Method	13.6	y = 2.6 + 0.91(x)	0.975
Reference	11.4		

D. **Specificity**

The cross-reactivity of the Insulin ELISA method to selected substances was evaluated by adding the interfering substance(S) to a serum matrix at the following concentration(s). The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of insulin needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Insulin	1.0000	–
Proinsulin	0.0078	100 ng/ml
C-Peptide	ND	75 ng/ml
Glucagon	ND	150 ng/ml

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

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Date Adopted	**2022-09**
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Brand Name	**AccuDiag™**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands.** www.cepartner4u.eu
Revision Date: 2019-07-16	