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Insulin ChLIA Kit

Catalog No.: 07M2475A

INTENDED USE

The MP Biomedicals Insulin ChLIA Kit is intended to be used for the quantitative determination of Insulin Concentration in Human Serum by a Microplate Chemiluminescence Assay (ChLIA). This test is for in vitro diagnostic use only.

SUMMARY AND EXPLANATION OF THE TEST

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. (Please see MP Biomedicals C-Peptide ChLIA Cat#07M2775A). These insulinomas can be localized by provocative intravenous doses of *tolbutamide* and *calcium*.

PRINCIPLE

Chemiluminescence immunoassay (Type 3)
The essential reagents required for a chemiluminescence assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin (S.Avidin) coated on the well and exogenously added biotinylated monoclonal anti-Insulin antibody (Ab).

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen (Ag), 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}-$^{Btn}Ab_{(M)}$ + $\underline{\text{Streptavidin}}_{CW} \Rightarrow$ $\underline{\text{Immobilized complex}}$
$\underline{\text{Streptavidin}}_{CW}$ = Streptavidin immobolized on well
$\underline{\text{Immobilized complex}}$ = sandwich complex bound to the well

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. The enzyme activity is determined by reaction with a light emitting substrate. 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

A. Insulin Calibrators – 2.0 mL/vial (Dried)
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 sixty (60) days at 2-8°C. 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 Tracer Reagent – 13 mL/vial
One (1) vial containing enzyme labeled affinity purified monoclonal mouse antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Light Reaction Wells 96 wells
One 96-well white 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/vial
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C (see Reagent Preparation Section).

E. Signal Reagent A – 7 mL/vial
One (1) bottle containing luminol in buffer. Store at 2-8°C (see Reagent Preparation Section).

F. Signal Reagent B – 7 mL/vial
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C (see Reagent Preparation Section).

G. Product Insert.

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

Required but not provided:
1. Pipette capable of delivering 0.050 mL (50 µL) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 mL (100 µL) and 0.350 mL (350 µL) volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200 µL) and (200-1000 µL) dispenser(s) for conjugate and substrate dilutions.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate luminometer
6. Test tubes for dilution of enzyme conjugate and substrate A and B.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
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, 1988, 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.

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 sample(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, 0.100 mL (100 µL) of the specimen is required

REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of Wash Solution Concentrate to 1000 mL with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2-8°C
   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 mL of A and 1mL of B per two (2) eight well strips (A slight excess of solution is made). Discard the unused portion if not used within 36 hours after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators 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 (50 µL) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 mL (100 µL) of the Insulin Tracer 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 60 minutes at room temperature
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350 mL (350 µL) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 four (4) additional times.**
8. Add 0.100 mL (100 µL) of working signal reagent solution to all wells (see Reagent Preparation Section**)**. **Always add reagents in the same order to minimize reaction time differences between wells. Do not shake the plate.**
9. Incubate at room temperature for five (5) minutes in the dark.
10. Read the RLU's (*Relative Light Units*) using a 96 well microplate luminometer for 0.2 – 1.0 seconds per well. **The results should be read within thirty (30) minutes of adding the working signal reagent.**

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated ranges for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control records should be maintained and used to monitor batch to batch consistency.

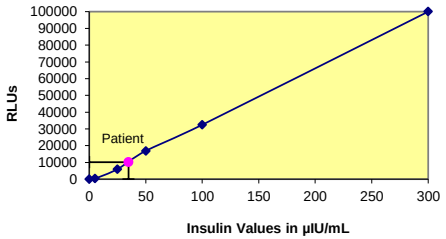
CALULATION AND RESULTS

A dose response curve is used to ascertain the concentration of insulin in unknown specimens.
1. Record the RLU's (Relative Light Units) obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLU's 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 RLU's 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 duplicates of the unknown may be averaged as indicated). In the following example, the average RLU's (10217) of the patient intersects the calibration curve at (34.7 µIU/mL) insulin concentration (See Figure 1)*.

Note 1: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLU's of the calibrators have been normalized to 100,000 RLU's for the F calibrator (greatest light output). This conversion eliminates differences cause by efficiency of the various instruments that can be used to measure light output.

Figure 1



EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (µIU/mL)
Cal A	A1	18	23	*0*
	B1	28		
Cal B	C1	407	401	*5*
	D1	394		
Cal C	E1	5983	6014	*25*
	F1	6044		
Cal D	G1	16955	16893	*50*
	H1	16832		
Cal E	A2	32659	32464	*100*
	B2	32269		
Cal F	C2	98734	100000	*300*
	D2	101266		
Ctrl 1	E2	270	282	***3.9***
	F2	294		
Ctrl 2	G2	41874	41974	***123.7***
	H2	42074		
Patient	A3	10290	10217	***34.7***
	B3	10144		

LIMITATIONS OF PROCEDURE

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 specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Patient samples with Insulin concentrations above 300 µIU/mL may be diluted with the zero calibrator and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from MP Biomedicals' 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 procedures 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 used 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, MP Biomedicals 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.

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 MP Biomedicals Insulin ChLIA test.

Each laboratory is advised to establish its own ranges for normal and abnormal populations. These ranges are always dependent upon locale, population, laboratory, technique and specificity of the method.

Based on the clinical data gathered 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 µU/mL
Diabetic (Type II)	0.7 – 25 µIU/mL

PERFORMANCE CHARACTERISTICS

Precision

The within and between assay precision of the Insulin ChLIA test were determined by analyses on two 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 Table 2 and Table 3.

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

Sample	N	X	σ	C.V.
Pool 1	24	7.86	0.60	7.6%
Pool 2	24	45.23	4.26	9.4%
Pool 3	24	133.92	5.69	4.2%

TABLE 3
Between Assay Precision* (Values in µIU/mL)

Sample	N	X	σ	C.V.
Pool 1	16	10.16	0.98	9.6%
Pool 2	16	45.53	3.48	7.7%
Pool 3	16	140.53	7.27	5.2%

*As measured in several experiments in duplicate.

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 0.114 µIU/mL.

Accuracy

The Insulin ChLIA test was compared with a reference microplate enzyme immunoassay (ELISA) assay. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from 0.01 µIU/mL – 132 IU/mL). The total number of such specimens was 105. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method (Y)	10.8	y =-0.8 + 0.953(x)	0.985
Reference (X)	11.2		

Only slight amounts of bias between the Insulin ChLIA test and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

Specificity

The cross-reactivity of the Insulin ChLIA test 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

High Dose Hook-Effect:

The test will not be affected by Insulin concentrations up to 10,000 µIU/mL in serum. However, samples expected to be over 300 µIU/mL should be diluted 1:10 and 1:100 in normal pooled human serum and the normal pool assayed alongside to obtain a base value. The base value and dilution factor should be taken into account to get the corrected concentration of Insulin in the sample.

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