Datasheet for ABIN649083

Insulin C-Peptide ELISA Kit

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Overview

Quantity:	96 tests
Target:	Insulin C-Peptide
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	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. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylatedantibody. 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. Immunoenzymometric assay: The essential reagents required for an immunoenzymometric 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. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylatedantibody. 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. dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	A. Insulin/C-Peptide COMBICAL™ Calibrators Insulin Calibrators (2. 0 ml/vial) (Dried). Six vials of references for Insulin and C-Peptide antigens at levels of 0(A), 5(B), 25(C), 50(D), 100(E), and 300(F) µIU/ml for Insulin and 0(A), 0. 2(B), 1. 0(C), 2. 0(D), 5. 0(E), and 10. 0(F) ng/ml for C-Peptide. Reconstitute each vial with 2ml of distilled or deionized water. The reconstituted calibrators are stable for 60 days at 2-8°C. A preservative has been added. The reconstituted calibrators are stable for 7 days at 2-8°C. In order to store for a longer period of time aliquot the reconstituted calibrators in cryo vials and store at -20°C. 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 for insulin and WHO 1st IRP 84/510 for C-Peptide. B. Enzyme-Insulin Enzyme Reagent (13ml/vial), antibody conjugate and Biotinylated monoclonal antibody (13 ml/vial). One vial containing enzyme labeled affinity purified monoclonal mouse antibodyx-insulin IgG, biotinylated monoclonal mouse x-insulin IgG in buffer, dye, and preservative. Store at 2-8C. C. C-Peptide Enzyme Reagent (13ml/vial). One vial containing enzyme labeled affinity purified monoclonal mouse antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C. D. Streptavidin Coated Microplate 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. E. Wash Solution Concentrate Concentrate (20 ml). One vial containing a

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	surfactant in phosphate buffered saline. A preservative has been added. Store at 2-30°C. F. Substrate A (7. 0ml/vial). One bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. G. Substrate B (7. 0ml/vial). One bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C. H. Stop Solution(8. 0ml/vial). One bottle containing a strong acid (1N HCl). Store at 2-30°C. I. Product Insert: (Instruction Booklet). Instructions: Note 1: Do not use reagents beyond the kit expiration date. Note 2: Opened reagents are stable for 60 days when stored at 2-8°C except for calibrators. Note 3: Above reagents are for a single 96-well microplate.
Material not included:	1. Pipette(s) capable of delivering 50µl and 100µl volumes with a precision of better than 1. 5%. 2. Dispenser(s) for repetitive deliveries of 0. 100ml and 0. 300ml volumes with a precision of better than 1. 5% (optional). 3. Microplate washer or a squeeze bottle (optional). 4. Microplate Reader with 450nm and 620nm wavelength absorbance capability. (The 620nm filter is optional). 5. Adjustable volume (200-1000µl) repete dispenser. 6. Container(s) for mixing of reagents (see below). 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. Storage container for storage of wash buffer. 12. Distilled or deionized water. 13. Quality Control Materials.

Target Details

Target:	Insulin C-Peptide
Abstract:	Insulin C-Peptide Products
Background:	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. Diabetes is one of the leading causes of disability and death in the U. S. It affects an estimated 16 million Americans, about one third of them do not even know they have the disease. The causes of diabetes are not precisely known, but both genetic and environmental factors play a significant role. The disease is marked by deficiencies in the body's ability to produce and properly use insulin. The most common forms of diabetes are type 1, in which the body's ability to produce insulin is destroyed, and type 2, in which the body is resistant to insulin even though some amount of

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insulin may be produced. In-vitro determination of insulin and C-Peptide levels help in the differential diagnosis of liver disease, acromegaly, Cushing's syndrome, familial glucose intolerance, insulinoma, renal failure, ingestion of accidental oral hypoglycemic drugs or insulin induced factitious hypoglycemia. Both insulin and C-Peptide are produced by enzymatic cleavage of proinsulin. Proinsulin is stored in the secretory granules of pancreatic (-cells and is split into a 31 amino acid connecting peptide (C-Peptide, MW 3600) and insulin (MW 6000). C-Peptide is devoid of any biological activity but appears to be necessary to maintain the structural integrity of insulin. Although insulin and C-Peptide are secreted into portal circulation in equimolar concentrations, fasting levels of C-Peptide are 5-10 fold higher than those of insulin owing to the longer half-life of C-Peptide. The liver does not extract C-Peptide however, it is removed from the circulation by degradation in the kidneys with a fraction passing out unchanged in urine. Hence urine C-Peptide levels correlate well with fasting C-Peptide levels in serum. The glucagon stimulated C-Peptide determination is often used for differential diagnosis of insulin-dependent from non-insulin-dependent diabetic patients. On the other hand the circulatory insulin can be found at much higher levels like 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. Intended Use: The test is intended to be used for the quantitative determination of insulin or C-Peptide levels in human serum. The test is for in vitro diagnostic use only. Q. C. Paramters: In order for the assay results to be considered valid the following criteria should be met. The absorbance (OD) of calibrators 0 µIU/ml should be lower than 0. 1. 2. The absorbance (OD) of calibrators 300 µIU/ml or 10ng/ml should be greater than 1. 23. . 3. Four out of six quality control pools should be within the established ranges.

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Application Notes: Precautions: 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.

Application Details

Sample Volume:	50 µL
Plate:	Pre-coated
Reagent Preparation:	1. Wash Buffer: Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C) for up to 60 days. 2. Working Substrate Solution: 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: Do not use the working substrate if it looks blue. AND STORAGE: 1. Wash Buffer: Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C) for up to 60 days. 2. Working Substrate Solution: 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: Do not use the working substrate if it looks blue. Pour the contents of the vial labeled Solution 'A' into the vial labeled Solution B. Mix and Store at 2-8°C. Use within 60 days. Or for longer periods of usage determine the amount of reagent needed and prepare by mixing equal portions of Substrate A and Substrate B in a suitable container. For example, add 1ml of A and 1ml of B per two eight well strips (A slight excess of solution is made. Discard the unused portion). Note: Do not use the working substrate if it looks blue. Note 1: Do not use reagents beyond the kit expiration date. Note 2: : Opened reagents are stable for 60 days when stored at 2-8°C. Note 3: If the substrate solution turns blue, discard the reagent.
Sample Collection:	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 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 repetitive freezing and thawing. When assayed in duplicate, 0. 100ml of the specimen is required. The specimens shall be blood serum 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 red-top venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8°C for a maximum period of Five days. If the specimen(s) cannot be

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	assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0. 100ml of the specimen is required.
Calculation of Results:	A dose response curve is used to ascertain the concentration of Insulin or C-Peptide in unknown specimens. 1. Record the absorbance obtained from the printout of the microplate reader. 2. Plot the absorbance for each duplicate serum reference versus the corresponding Insulin or C-Peptide concentration in µIU/ml or ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting). Draw the best-fit curve through the plotted points. To determine the concentration of Insulin or C-Peptide 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 duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0. 624) (0. 433) intersects the dose response curve at 66. 8 µIU/ml (1. 03ng/ml) for the Insulin (C-Peptide) concentration. Average the absorbance of each duplicate. Plot the mean absorbance for each duplicate calibrator versus the corresponding Insulin concentration in µIU/ml on linear graph paper. 3. Draw a Dose Response Curve (DRC) using 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 duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0. 274) intersects the DRC at (22. 3µIU/ml Insulin concentration (See Figure 1). Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction.
Restrictions:	For Research Use only

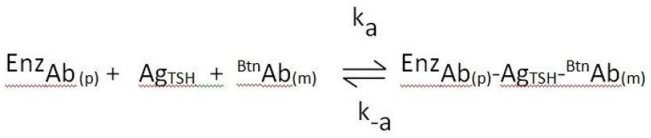
Handling

Handling Advice:	Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C). 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 or C-Peptide 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

Handling

	with absorbent paper. 7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two additional times for a total of three 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 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 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 30 minutes of adding the stop solution.
Storage:	4 °C/-20 °C

Images



Btn Ab (m) = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag TSH = Native Antigen (Variable Quantity)
Enz Ab (p) =Enzyme -Polyclonal Antibody (Excess Quantity)
Enz Ab (p)-Ag TSH-Btn Ab (m) = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Image 1. The essential reagents required for an immunoenzymometric 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 coated on the well and exogenously added biotinylated monoclonal anti-TSH 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 equation in Figure 1.

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

2.

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.