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Product Information

C-Peptide ELISA kit

Catalog Number: EA100869
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The C-peptide ELISA kit is intended for the quantitative determination of human C-peptide levels in human serum.

Background

Human C-Peptide has a molecular mass of approximately 3000 daltons. C-Peptide has no metabolic function. However, since C-Peptide and insulin are secreted in equimolar amounts, the immunoassay of C-Peptide permits the quantitation of insulin secretion. This is the reason for the clinical interest of serum or plasma determinations of C- Peptide. Moreover, C-Peptide measurement has several advantages over immunoassays of insulin. The half-life of C- Peptide in the circulation is between two and five times longer than that of insulin. Therefore, C-Peptide levels are a more stable indicator of insulin secretion than the more rapidly changing levels of insulin. A very clear practical advantage of C-Peptide measurement arising from its relative metabolic inertness as compared to insulin is that C- Peptide levels in peripheral venous blood are about 5-6 times greater than insulin levels. Also, relative to an insulin assay, the C-Peptide assay's advantage is its ability to distinguish endogenous from injected insulin. C-Peptide has also been measured as an additional means for evaluating glucose tolerance and glibenclamide glucose tests. C- Peptide levels are in many ways a better measurement of endogenous insulin secretion than peripheral insulin levels. C-Peptide may be measured in either blood or urine. With improved sensitive C-Peptide immunoassays, it is now possible to measure C-Peptide values at extremely low levels. The clinical indications for C-Peptide measurement include diagnosis of insulinoma and differentiation from factitious hypoglycemia, follow-up of pancreatectomy, and evaluation of viability of islet cell transplants. Recently, these indications have been dramatically expanded to permit evaluation of insulin dependence in maturity onset diabetes mellitus

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Principle of the Test

The C-Peptide is a solid phase direct sandwich ELISA method. The samples and conjugate reagent (anti C-peptide biotin & HRP) are added to the wells coated with Streptavidin. C-peptide in the patient’s serum binds to the matched pair Abs, forming a sandwich complex and simultaneously the complex is being immobilized on the plate through streptavidin-biotin interactions. Unbound proteins and HRP conjugate is washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of C-peptide in the samples. A standard curve is prepared relating color intensity to the concentration of the C-Peptide.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. Standards (1-6) 6 vials, lyophylized	Reconstitute with 1 ml DH2O
3. C-peptide Conjugate Reagent (ready to use)	12 ml
4. TMB Substrate (ready to use)	12 ml
5. Stop Solution (ready to use)	12 ml
6. Wash Solution 20x	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8 °C) for 2 days. If storage time exceeds 2 days, store frozen at -20 °C for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly hemolyzed or lipemic specimens.

Reagent Preparation

1. **Standards:** Reconstitute the lyophilized standards with 1 ml distilled water. Allow them to remain undisturbed until completely dissolved, and then mix well by gentle inversion.
   The reconstituted standards are stable for 24 hours when stored sealed at 2-8 °C. To assure maximum stability of the reconstituted standards, aliquot the standards and store at -20 °C. Do not freeze-thaw more than once.
2. **Prepare 1X Wash buffer**: Add the Wash Buffer (20x, 25 ml) to 475 ml of distilled or de-ionized water. Store at room temperature (20-25°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control, and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Format the microplate wells for each reference, 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 50 µl of the appropriate standard, control, or specimen into the assigned well.
3. Pipette 100 µl C peptide conjugate reagent into each well.
4. Cover the plate and incubate for 60 minutes at room temperature (20-25 °C).
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1x wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

Calculation of Results

The standard curve is constructed as follows:

1. Check C-Peptide standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the OD (Optical Density) for each Total C-Peptide standard point (Y-axis) versus the Total C-Peptide standard concentrations (X-axis) on a linear graph paper. Draw the best curve through the points.
3. Read the concentration (ng/ml) for controls and each unknown sample from the curve. Record the value for each control or unknown sample
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

Example of Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Conc.ng/mL	OD450
Std 1	0	0.01
Std 2	0.15	0.03
Std 3	0.75	0.15
Std 4	2.0	0.4
Std 5	6	1.29
Std 6	10.0	2.26

References

1. Ashby, J. and Frier, B.: Circulating C-Peptide: Measurement and Clinical Applications. Annals of Clinical Biochemistry. 18:125, 1981.
2. Beischer, W.: Proinsulin and C-Peptide in Humans. Hormones in Normal and Abnormal Human Tissues. Volume 3K, Fotherby and Pal, S., ed. (Berlin: Walter DeGruyter). pp. 1-43, 1983
3. Beyer, J., Krause V., Cordes V.: C-Peptide: Its Biogenesis, Structure, Determination and Clinical Significance. Giornale Italiano di Chimica Clinica 4 Supp. 9:22, 1979

4. Bonger, A. and Garcia-Webb, P.: C-Peptide Measurement: Methods and Clinical Utility. CRC Critical Reviews in Clinical Laboratory Sciences. 19:297, 1984.
5. Blix, P. Boddie-Wills, C., Landau, R., Rochman, H. Rubenstein, A.: Urinary C-Peptide: An Indicator of Beta-Cell Secretion under Different Metabolic Conditions. Journal of Clinical Endocrinology and Metabolism. 54:574, 1982.
6. Rendell, M.: C-Peptide Levels as a Criterion in Treatment of Maturity-Onset Diabetes. Journal of Clinical Endocrinology and Metabolism. 57 (6): 1198, 1983
7. Horwitz, D., et al.: Proinsulin, Insulin and C-Peptide concentrations in Human Portal and Peripheral Blood. Journal of Clinical Investigation. 55:1278, 1975

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