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C-Peptide ELISA

Catalog No. CP441S (96 Tests)

INTENED USE

The C-peptide ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human C-peptide levels in human serum.

SUMMARY AND EXPLANATION

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.

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.

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. C-peptide Standards: 6 vials (lyophilized)	Lyophilized, Recon. with 1.0 ml DH_2O
3. C-peptide Conjugate Reagent: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12 ml
5. Stop Solution: 1 bottle (ready to use)	12 ml
6. 20X Wash concentrate: 1 bottle	25 ml

MATERIALS NOT PROVIDED

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

STORAGE AND STABILITY

1. Store the kit at 2 – 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The standard set contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. For research use only. Not for use in diagnostic procedures.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION PREPARATION

1. **Serum:**
   a) Collect blood specimens and separate the serum immediately.
   b) 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.
   c) Avoid multiple freeze-thaw cycles.
   d) Prior to assay, frozen sera should be completely thawed and mixed well.
   e) Do not use grossly lipemic specimens.

REAGENT PREPARATION

Standards: Reconstitute the lyophilized standards with 1.0 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.

Wash Buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature.

Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 50 µl of C-peptide standards, control and patient's sera onto appropriate wells.
3. Add 100 µl of C-peptide conjugate reagent to all wells.
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 for each C-Peptide standard point (vertical axis) versus the C-Peptide standard concentrations (horizontal 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

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.

C-peptide (ng/mL)	Absorbance (450nm)
0	0.01
0.15	0.03
0.75	0.15
2	0.40
6	1.29
10	2.26

LIMITATION OF THE PROCEDURE

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.