


REFERENCES

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C-Peptide ELISA

Catalog No. CP441S (96 Tests)

INTENDED USE

The C-peptide ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human C-peptide levels in human serum. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

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. C-peptide is released during insulin biosynthesis. Research uses include beta-cell function studies, insulin secretion regulation, and metabolic disorder models such as type 1 and type 2 diabetes.

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 ₂ O
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



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WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The standards 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. 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

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

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.