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Product Information

C-Peptide ELISA

Catalog Number **SE120040**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Human C-Peptide has a molecular mass of ~3,000 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 advantage of the C-Peptide assay 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. 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.

The C-Peptide ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human C-peptide levels in human serum. The C-Peptide ELISA kit is a solid phase direct sandwich ELISA method.

The standards, samples, and controls are added into the selected wells coated with anti C-Peptide monoclonal anybody. C-Peptide in the standards, controls, and serum samples binds to anti-C-Peptide Ab on the wells. Unbound protein is washed off by wash buffer. The anti-C-Peptide-HRP conjugated second antibody is added and then binds to C-Peptide. Unbound proteins and HRP conjugate is washed off by wash buffer. Upon the addition of the substrate, the enzyme activities are 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.

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

Components

Materials Provided	96 Tests
Microwells coated with anti-C-peptide Ab	12 × 8 × 1
Standards (1-6) 6 vials, lyophylized	Reconstitute with 2 ml of distilled water
Enzyme Conjugate (Ready to use)	12 ml
TMB Solution	12 ml
Stop Solution	12 ml
Wash Solution 20×	25 ml

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Serum Sample Preparation
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 lipemic specimens.

Preparation of Standards
Reconstitute the lyophilized standards with 2.0 ml of distilled water. Allow them to remain undisturbed until completely dissolved and then mix well by gentle inversion.

20× Wash Buffer Concentrate
Prepare 1× wash buffer by adding the contents of the bottle to 475 ml of distilled water. Store 1× wash buffer at room temperature.

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes:
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.

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.

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18–26 °C).

1. Format the microplate wells for each reference, control, and serum sample 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 sample into the assigned well.
3. Pipette 100 µl of Enzyme Conjugate into each well.
4. Gently mix plate for 15–20 seconds.
5. Incubate for 60 minutes at room temperature.
6. Remove liquid from all wells. Wash wells 3 times with 300 µl of 1× wash buffer (see Preparation Instructions). Blot on absorbent paper towels.
7. Add 100 µl of TMB Substrate to all wells.
8. Incubate for 15 minutes at room temperature.
9. Add 50 µl of Stop Solution to each well and gently mix for 15–20 seconds.
10. Read the absorbance on ELISA Reader of each well at 450 nm within 15 minutes after adding the Stop Solution.

Results

Calculations

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 the value is checked on every kit.
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.

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.

Standard	Optical Units
Standard 1 (0 ng/ml)	0.014
Standard 2 (0.2 ng/ml)	0.044
Standard 3 (1.0 ng/ml)	0.219
Standard 4 (2.0 ng/ml)	.527
Standard 5 (5.0 ng/ml)	1.656
Standard 6 (10 ng/ml)	3.042

3. Read the concentration (ng/ml) for controls and each unknown sample from the curve. Record the value for each control or unknown sample

Expected Values

It is recommended that each laboratory establish its own range of normal C-Peptide level. The normal range of values observed with C-Peptide ELISA kit with normal adult males and females are as follows:

	n	Mean±2 SD
Adult (Serum)	30	0.5–3.0 ng/ml

C-Peptide levels have been shown to increase after intake of glucose by 100–600 %.

Note: 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.

Product Profile

Sensitivity

The sensitivity of the assay is 0.013 ng/ml. The sensitivity was determined by calculating the mean plus 2 SD of the standard zero point tested 20 times in the same run.

Correlation with a Reference ELISA kit:

A total of 60 samples were tested by this kit and a commercially available C-peptide ELISA kit.
The linear regression curve was calculated as:
Y = 0.838 + 0.05, r = 0.991.

Precision

Intra-Assay

Serum	Number of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	20	2.82	0.101	3.6
2	20	5.34	0.191	3.9
3	20	7.48	0.21	2.6

Inter-Assay

Serum	Number of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	16	2.51	0.271	10.8
2	16	5.00	0.347	6.9
3	16	6.81	0.666	9.8

Linearity

Two different serum samples were diluted with the "0" calibrator to 1:2, 1:4, and 1:8. C-Peptide values were calculated and results were corrected with the dilution factor.

Original Value		Percentage of Recovery		
Serum	(ng/ml)	1/2	1/4	1/8
1	10	97.6	108.8	109.4

Recovery

Expected value (ng/ml)	Recovered (ng/ml)	Percentage of Recovery
3.20	3.28	102.6
4.38	4.51	103.0
8.37	8.88	106.2

References

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