



C-Peptide ELISA Kit

(Catalog # E4701-100; 96 assays ; Storage at 2-8°C)

rev 06/19

I. Introduction:

C-peptide serves as an important linker between A-chain and B-chain of insulin and facilitates the efficient assembly, folding, and processing of insulin in the endoplasmic reticulum. Equimolar amounts of C-peptide and insulin are stored in secretory granules of the pancreatic beta cells and both are eventually released to the portal circulation. The sole interest in C-peptide was as a marker of insulin secretion. Newly diagnosed diabetes patients often get their C-peptide levels measured as a means of distinguishing type 1 and type 2 diabetes. C-peptide is also used for determining the possibility of gastrinomas associated with Multiple Endocrine Neoplasm syndromes (MEN 1).

II. Sensitivity:

0.2 ng/ml

III. Sample Type:

- Serum

IV. Kit Contents:

Components	E4787-100	Part Number
Microwells coated with anti-C-peptide Ab	12 strips x 8 wells	E4701-100-1
Standards (S1-S6) 6 vials	lyophilized	E4701-100-2
Enzyme Conjugate (Ready to use)	12 ml	E4701-100-3
TMB Solution	12 ml	E4701-100-4
Stop Solution	12 ml	E4701-100-5
Wash Solution 20X	25 ml	E4701-100-6

V. User Supplied Reagents and Equipment:

- Distilled or deionized water,
- Microplate reader capable of reading absorbance at 450 nm

VI. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

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).

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

VII. Assay Protocol:

1. Sample preparation: Collect blood specimens and separate the serum immediately. 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. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. **Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activity.**

2. Assay Procedure:

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

1. Pipette 50µl of the appropriate standard, control or specimen into the assigned well.
2. Pipette 100µl Enzyme Conjugate into each well.
3. Gently mix plate for 15-20 seconds.
4. Incubate for 60 minutes at room temperature.
5. Remove liquid from all wells. Wash wells three times with 300 ul of 1X wash buffer (see Reagent Preparation Section). 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 each well and gently mix for 15-20 seconds.
9. Read the absorbance on ELISA Reader of each well at 450 nm within 15 minutes after adding the stop solution.

3. Calculation:

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:

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

VIII. Related Products:

C-reactive/CRP Monoclonal Antibody (A1208)

FOR RESEARCH USE ONLY! Not to be used on humans.