Version 02-05/25

User's Manual

Human Ultra Sensitive C-peptide ELISA Kit

REF **DEIA-CL003U**

Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This ultra-sensitive C-peptide ELISA kit is intended for use in the quantitative determination of low pico gram level of human connecting peptide in serum or plasma.

General Description

C-peptide is a small 31-amino acid peptide usually produced in the pancreas as a byproduct of the cleavage of proinsulin in the synthesis of insulin. Proinsulin consists of A and B chain and connecting peptide in the middle, called C-peptide. It is generally found in equimolar amounts to insulin in circulation. Since the half- life of C-peptide is 3-4 times that of insulin, it serves as a useful measure of insulin production in the beta cells of the pancreas. Testing for C-peptide levels can help find the cause of low blood sugar (hypoglycemia) aid in distinguishing type 1 from type 2 diabetes. A person with diabetes may have a normal level of C- peptide which indicates the body is making plenty of insulin but the body is just not responding properly to it. This is the hallmark of type 2 diabetes (adult insulin-resistant diabetes). Some studies have suggested that C-peptide may have chemotactic effects on the inflammatory cells and might have a role in increased risk of atherosclerosis in persons with type-2 diabetes.

Principles of Testing

This ELISA kit is designed, developed and produced for the quantitative measurement of human C-peptide in serum and/or EDTA-plasma samples. The assay utilizes the "sandwich" technique with selected antibodies that bind to various epitopes of C-peptide.

Assay standards, controls and patient samples are added directly to wells of a microplate that is coated with an anti-human C-peptide specific antibody. Simultaneously, a horseradish peroxidase- conjugated monoclonal C-peptide specific antibody is added to each well. After the first incubation period, the antibody on the wall of microtiter well captures human C-peptide in the sample and unbound proteins in each microtiter well are washed away. A "sandwich" of "anti-C-peptide antibody --- human C-peptide --- HRP conjugated tracer antibody" is formed. The unbound tracer antibody is removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to human C-peptide on the wall of the microtiter well is directly proportional to the amount of C-peptide in the sample. A standard curve is generated by plotting the absorbance versus the respective human C-peptide concentration for each standard on point-to-point or 4 parameter curve fit. The concentration of human C-peptide in test samples is determined directly from this standard curve.

Reagents And Materials Provided

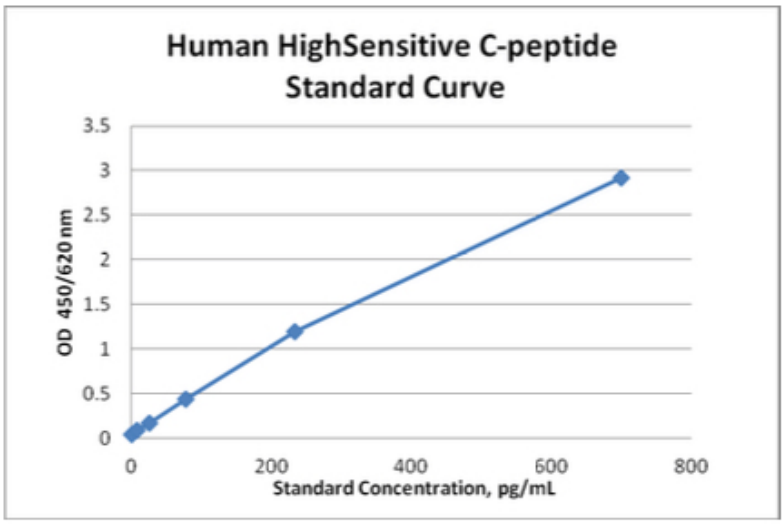
1. x Microplate 96T
2. 1 x Anti-C-Peptide Antibody-HRP 6mL
3. 1 x Wasn Concentrate 30mL
4. 1 x TMB 12mL

5. 1 x Stop Solution 12mL
6. x Huam C-peptide Standard
7. 2 x Human C-Peptide Control

Storage

2-8°C

Typical Standard Curve



Precision

The intra-assay precision was validated by measuring three control samples with 16 replicate determinations.

Sample #	Mean C-peptide Value (pg/mL)	CV (%)
1	396.6	6.9
2	199.7	6.1
3	217.7	6.9

The inter-assay precision was validated by measuring two control levels in duplicate in 12 individual assays.

Sample #	Mean C-peptide Value (pg/mL)	CV (%)
1	49.3	4.5
2	162.3	7.1

Sensitivity

0.57 pg/mL.

Linearity

Two **EDTA plasma** samples were collected, diluted with standard zero matrix and tested. The results of C-peptide percent recovery value in pg/mL are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	RECOVERY %
Neat A	155.6	-
1:2	69.2	89.0
1:4	35.8	92.0
1:8	18.6	95.6
Neat B	180.4	-
1:2	96.6	107.1
1:4	46.4	102.9
1:8	23.8	105.4

Two **serum** samples were collected, diluted with standard zero matrix and tested. The results of C-peptide percent recovery value in pg/mL are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	RECOVERY %
Neat A	145.574	-
1:2	66.261	91.0
1:4	36.684	100.8
1:8	16.499	90.7
Neat B	182.5	-
1:2	80.0	87.7
1:4	45.0	98.6
1:8	24.3	106.7

Recovery

Two **EDTA plasma** samples and three assay standards (26.0, 77.8 and 233.3 pg/mL) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	RECOVERY %
Neat A	180.4	-
Std-2: 26.0 pg/mL	86.8	84.1
Std-3: 77.8 pg/mL	122.7	95.0
Std-4: 233.3 pg/mL	200.4	96.9
Neat B	136.0	-
Std-2: 26.0 pg/mL	88.2	100.3
Std-3: 77.8 pg/mL	120.3	100.5
Std-4: 233.3 pg/mL	200.2	98.9

Two **serum** samples and three assay standards (26.0, 77.8 and 233.3 pg/mL) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	RECOVERY %
Neat A	127.9	-
Std-2: 26.0 pg/mL	85.3	101.8
Std-3: 77.8 pg/mL	125.5	108.5
Std-4: 233.3 pg/mL	194.2	98.1
Neat B	182.5	-
Std-2: 26.0 pg/mL	88.2	84.6
Std-3: 77.8 pg/mL	111.7	85.8
Std-4: 233.3 pg/mL	209.3	100.7