

EDI™ Human Ultra-Sensitive C-Peptide ELISA Enzyme Linked ImmunoSorbent Assay (ELISA) for the Quantitative Measurement of Human Connecting Peptide Levels in blood



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.

SUMMARY OF PHYSIOLOGY

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.

ASSAY PRINCIPLE

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: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use, allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

- 1. Anti-C-peptide Antibody Coated Microplate (Cat. No. 30693)**
One microplate with 12 by 8 strips (96 wells total) coated with anti-human C-peptide antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.
- 2. HRP Conjugated Anti-C-peptide Antibody (Cat. No. 30697)**
One vial containing 0.5 mL HRP-labeled C-peptide antibody in a stabilized protein matrix. This reagent should be diluted with C-peptide Tracer Antibody Diluent and should be stored at 2-8°C. It is stable until the expiration date on the kit box.
- 3. ELISA Wash Concentrate (Cat. No. 10010)**
One bottle containing 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.
- 4. ELISA HRP Substrate (Cat. No. 10020)**
One bottle containing 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.
- 5. ELISA Stop Solution (Cat. No. 10030)**
One bottle containing 12 mL of stop solution. This reagent may be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.
- 6. Human C-peptide Standards (Cat. No. 30761 – 30766)**
Six vials containing recombinant human C-peptide in a lyophilized bovine serum-based matrix with a non-azide preservative. **Refer to the vials for exact concentration of the standard.** These standards should be stored at 2 – 8°C and are stable until the expiration date on the kit box. Refer to assay procedure section for reconstitution instructions.
- 7. Human C-peptide Controls (Cat. No. 30766 – 30767)**
Two vials containing human C-peptide in a lyophilized bovine serum based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box. Refer to assay procedure section for reconstitution instructions.

8. Tracer Antibody Diluent (Cat. No. 30710)

One bottle containing **10 mL** ready-to-use buffer. It should be used only for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

SAFETY PRECAUTIONS

The reagents must be used in professional laboratory. Source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 100 μL .
2. Disposable pipette tips suitable for above volume dispensing.
3. Aluminum foil.
4. Deionized or distilled water.
5. Plastic microtiter well cover or polyethylene film.
6. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
7. Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm.

SPECIMEN COLLECTION

Serum and EDTA-plasma samples are suitable specimens for human C-peptide measurement. Only **100 μL** of human sample is required for a duplicate determination of human C-peptide with this test kit. No special preparation of individual is necessary prior to specimen collection. Samples should be collected by standard technologies of clinical laboratory practice and recommended by manufacturer of sample collection tube. It is extremely important to carefully separate the serum and plasma from blood cells to avoid hemolysis, etc. Serum/EDTA-plasma should be transferred to a clean test tube right after centrifugation. Human samples should be stored at 2 – 8°C if the assay is to be performed within 72 hours. Otherwise, patient samples should be stored at –20°C or below until measurement. Avoid more than three times freeze-thaw cycles of specimen. Do not use hemolyzed, hyperlipemic, heat-treated or any contaminated specimens.

ASSAY PROCEDURE

1. Reagent Preparation

- (1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
- (2) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.
- (3) Reconstitute assay standards and controls by adding **0.5 mL** of demineralized water to each standard and control bottle. Allow the standards and controls to sit undisturbed for 5 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solid is dissolved completely prior to use. These reconstituted standards and controls may be stored at 2- 8°C for up to **3 days** or below –20°C for **long-term** storage. Do not exceed 3 freeze-thaw cycles.
- (4) Prepare Tracer Antibody working solution by **1:21** fold dilution of the HRP Conjugated Anti-C peptide Antibody

(Cat. 30697) by adding the tracer antibody into the Tracer Antibody Diluent (Cat. 30710). Following is a table that outlines the relationship of strips used and antibody mixture prepared. **NOTE: the tracer antibody should be prepared just prior to use.**

Dilution Scheme	Tracer Antibody Diluent	Tracer Antibody
1	0.5 mL	25 μL
2	1 mL	50 μL
3	1.5 mL	75 μL
4	2 mL	100 μL
5	2.5 mL	125 μL
6	3 mL	150 μL
7	3.5 mL	175 μL
8	4 mL	200 μL
9	4.5 mL	225 μL
10	5 mL	250 μL
11	5.5 mL	275 μL
12	6 mL	300 μL

(5) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3	STRIP 4
A	STD 1	STD 5	SAMPLE 1	SAMPLE 5
B	STD 1	STD 5	SAMPLE 1	SAMPLE 5
C	STD 2	STD 6	SAMPLE 2	SAMPLE 6
D	STD 2	STD 6	SAMPLE 2	SAMPLE 6
E	STD 3	C 1	SAMPLE 3	
F	STD 3	C 1	SAMPLE 3	
G	STD 4	C 2	SAMPLE 4	
H	STD 4	C 2	SAMPLE 4	

- (6) Place a sufficient number of Anti-C-peptide antibody-coated microwell strips (Cat. 30693) in a holder to run human C-peptide standards, controls and unknown samples in duplicates.

2. Assay Procedure:

- (1) Add **50 μL** of Standards, Controls and patient samples into the designated microwells.
- (2) Add **50 μL** of the above diluted Tracer Antibody working solution to each well.
- (3) Seal the plate wells securely, cover with foil or similar material to protect from light. Incubate the plate static, at room temperature for **2 hr. \pm 5 minutes**.
- (4) Wash each well 5 times by dispensing 350 μL of working wash solution into each well, and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (5) Add **100 μL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
- (6) Cover the plate with aluminum foil or similar material to avoid exposure to light. Incubate the plate static, at room temperature for **20 minutes**.
- (7) Immediately add **100 μL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
- (8) Read the absorbance at 450 nm with reference filter at 620 nm or 650 nm.

PROCEDURAL NOTES

1. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
8. If adapting this assay to automated ELISA system such as DS-2 (Diamedix Corp., Miami), a procedural validation is necessary if there is any modification of the assay procedure.

INTERPRETATION OF RESULTS

It is recommended to use a point-to-point or 4-parameter standard curve fitting.

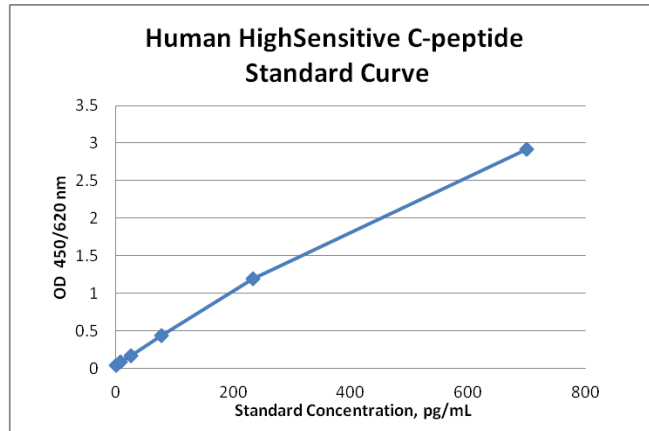
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the level 1 standard (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The human C-peptide concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance.

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from this Human C-peptide ELISA are represented. **This curve should not be used in lieu of standard curve generated with each assay.**

Well I.D.	OD 450/620 nm Absorbance			Results pg/mL
	Readings	Average	Corrected	
Std-1: 0 pg/mL	0.039 0.041	0.040	0	
Std-2: 8.6 pg/mL	0.084 0.083	0.083	0.043	
Std-3: 26.0 pg/mL	0.174 0.175	0.174	0.134	
Std-4: 77.8 pg/mL	0.446 0.437	0.442	0.402	
Std-5: 233.3 pg/mL	1.158 1.228	1.193	1.153	
Std-6: 700 pg/mL	2.991 2.845	2.918	2.878	
Control 1	0.360 0.392	0.376	0.336	65.1
Control 2	0.928 0.957	0.942	0.902	181.5



EXPECTED VALUES

Human non-fasting samples from normal healthy adults ages 20 – 60 were collected and measured with this ELISA. The recommended normal high cut-off for C-peptide concentration by using this ELISA is 5 ng/mL with an average level of 1.3 ng/mL (range 0.13 – 4.6 ng/mL, SD 0.94 ng/mL). We strongly recommend for each clinical laboratory to establish its own normal range by measuring EDTA plasma and/or serum with this ELISA.

LIMITATION OF THE PROCEDURE

1. An abnormally high C-peptide test result cannot be independently used for clinical diagnosis. As with other laboratory tests, a variety of analytical and pre-analytical factors may lead to false high test results. Physicians must interpret the test result in the light of each patient's clinical findings.
2. For sample values reading greater than the highest standard, it is recommended to re-assay samples with further dilutions (i.e. 1:10 or 1:100 with 5%BSA in 0.01M PBS).
3. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls.

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity (LLOD) of the C-peptide ELISA as determined by the 95% confidence limit on 16 duplicate determination of zero standard is approximately 0.57 pg/mL.

High Dose "hook" effect

This assay has showed that it did not have any high dose "hook" for C-peptide levels up to 150 ng/mL.

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

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

Spike 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


WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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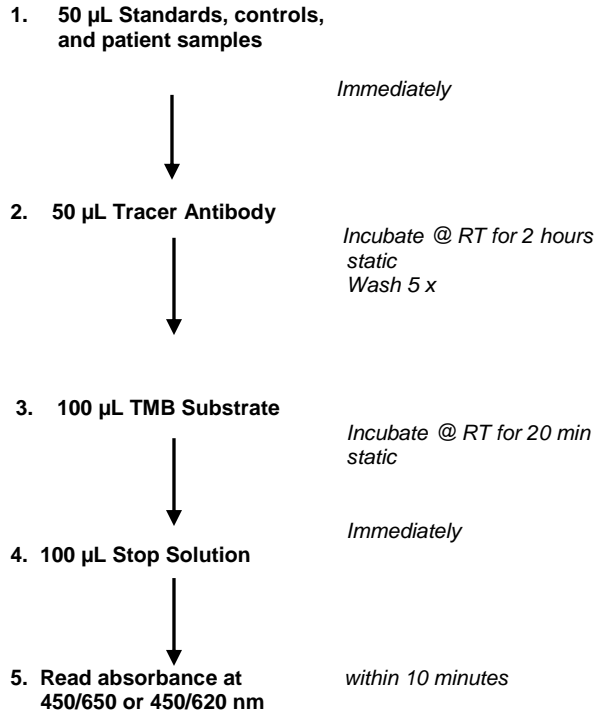
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Ultra Sensitive C-peptide ELISA: Condensed Assay Protocol



 Manufacturer	 No. of tests
 Catalog Number	 Keep away from heat and direct sun light
 Concentrate	 Store at
 In Vitro Diagnostic Device	 Use by
 Read instructions before use	 Lot No.
 Authorized Representative In Europe	