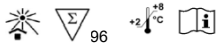


EDI™ Human C-peptide ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Quantitative Measurement of Human Connecting Peptide Levels in serum/plasma

REF KT-881 CE IVD 

INTENDED USE

This human C-peptide ELISA kit is intended for use in the quantitative determination of human connecting peptide in serum and/or plasma. This kit is for in vitro diagnostic use only.

SUMMARY OF PHYSIOLOGY

C-peptide is a small 31-amino acid peptide usually produced in the beta cell of 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 equal 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). For patient of type 1 diabetes treated with insulin, measuring C-peptide level is useful in evaluating beta cell function related to synthesis and release endogenous insulin into the circulation.

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 calibrators, 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 the microtiter well captures human C-peptide in the sample. A "sandwich" of "anti-C-peptide antibody --- human C-peptide --- HRP conjugated tracer antibody" is formed. The unbound tracer antibodies and other matrix protein from the test sample are 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 calibration curve is generated by plotting the absorbance versus the respective human C-peptide concentration for each calibrator on point-to-point or 4 parameter curve fit. The concentration of human C-peptide in test samples is determined directly from this calibration 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-human C-peptide Antibody Coated Microplate (30693)

Coated with anti-human c-peptide antibody.

Qty: 1 x 96 well microplate

Storage: 2 – 8°C

Preparation: Ready to Use

2. HRP Conjugated C-peptide Antibody (30697)

HRP-labeled C-peptide antibody in a stabilized protein matrix.

Qty: 1 x 12 mL

Storage: 2 – 8°C

Preparation: Ready to Use

3. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty: 1 x 30 mL

Storage: 2 – 25°C

Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

4. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 12 mL

Storage: 2 – 8°C

Preparation: Ready to Use

5. ELISA Stop Solution (10030)

0.5 M sulfuric acid

Qty: 1 x 12 mL

Storage: 2 – 25°C

Preparation: Ready to Use

6. Human C-peptide Calibrators Levels 1 to 5 (30741 - 30745)

Human C-peptide in a lyophilized bovine serum based matrix with a non-azide preservative. Refer to vials for concentration.

Qty: 5 x Vials

Storage: 2 – 8°C (Lyophilized), <-20°C (Reconstituted)

Do not exceed 3 freeze-thaw cycles.

Preparation: Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

7. Human C-peptide Controls (30746, 30747)

Human C-peptide in a lyophilized bovine serum based matrix with a non-azide preservative. Refer to vials for concentration.

Qty: 2 x Vials

Storage: 2 – 8°C (Lyophilized), <–20°C (Reconstituted)

Do not exceed 3 freeze-thaw cycles.

Preparation: Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic use only. Source material which contains reagents of bovine serum albumin 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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 & STORAGE

Serum and EDTA-plasma samples are suitable specimens for human C-peptide measurement. Only 50 µL of human sample is required for a duplicate determination of human C-peptide with this test kit. No special preparation of the individual is necessary prior to specimen collection. Samples should be collected by standard technologies of clinical laboratory practices and recommended by the manufacturer of the sample collection tubes. 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 immediately following 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 (10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.
3. Reconstitute assay calibrators and controls by adding 0.5 mL of demineralized water to each calibrator and control vial. Allow the calibrators 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 calibrators 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. Assay Procedure

1. Place a sufficient number of microwell strips (30693) in a holder to run calibrators (30741 – 30745), controls (30746, 30747), and samples in duplicate.

2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
C	Calibrator Level 2	Control 1	SAMPLE 3
D	Calibrator Level 2	Control 1	SAMPLE 3
E	Calibrator Level 3	Control 2	SAMPLE 4
F	Calibrator Level 3	Control 2	SAMPLE 4
G	Calibrator Level 4	SAMPLE 1	SAMPLE 5
H	Calibrator Level 4	SAMPLE 1	SAMPLE 5

3. Add 25 µL of calibrators (30746), controls (30746, 30747), and samples into the designated microwells.
4. Add 100 µL of the antibody (30697) into each microwell.
5. Cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 °C) with shaking at 400 to 450 rpm for 60 minutes.
6. Remove the plate sealer. Aspirate the contents of each microwell. Wash each well 5 times by dispensing 350 µL of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
7. Add 100 µL of substrate (10020) into each microwell.
8. Gently mix, and cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 °C) for 20 minutes.
9. Add 100 µL of stop solution (10030) into each microwell and mix gently.
10. Read the absorbance at 450/620 or 450/650 nm within 10 minutes with a microplate reader.

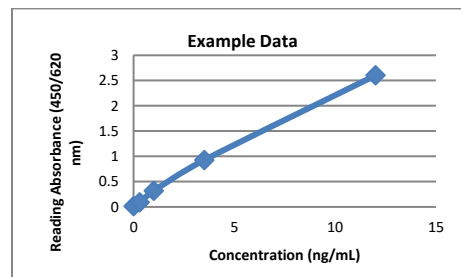
PROCEDURAL NOTES

1. It is recommended that all calibrators, 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. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.
9. If adapting this assay to automated ELISA system such as DS-2 or DSX (Dynex, USA), a procedural validation is necessary if there is any modification of the assay procedure.

INTERPRETION OF RESULTS

1. It is recommended to use a point-to-point or 4-parameter calibration curve fitting.
2. Calculate the average absorbance for each pair of duplicate test results.
3. Subtract the average absorbance of the level 1 calibrator (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
4. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator 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.
5. The human C-peptide concentrations for the controls and the patient samples are read directly from the calibration curve using their respective corrected absorbance.

Calibrator Level 5: 12 ng/mL	2.628	2.601	2.585	
	2.574			
Control 1	0.166	0.212	0.196	0.68
	0.257			
Control 2	0.652	0.576	0.576	2.07
	0.500			



LIMITATIONS 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 unknown sample value read directly from the assay that is greater than the highest calibrator, it is recommended to measure a further diluted sample for more accurate measurement.

QUALITY CONTROL

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

EXPECTED VALUES

Human **non-fasting** samples from normal healthy adults ages 20 – 60 were collected and measured with this ELISA. The average of C-peptide concentration by using this ELISA is 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 (fasting and non-fasting) by measuring EDTA plasma and/or serum with this ELISA.

$$\text{C-peptide (pmol/L)} = \text{C-peptide (ng/mL)} / 331$$

EXAMPLE DATA

A typical absorbance data and the resulting calibration curve from are represented.

Note: This curve should not be used in lieu of calibration curve run with each assay.

Well ID	Reading Absorbance (450/620nm)			Concentration (ng/mL)
	Readings	Average	Corrected	
Calibrator Level 1: 0 ng/mL	0.012	0.016	0.000	
	0.020			
Calibrator Level 2: 0.3 ng/mL	0.094	0.089	0.073	
	0.085			
Calibrator Level 3: 1.0 ng/mL	0.290	0.316	0.300	
	0.342			
Calibrator Level 4: 3.5 ng/mL	0.998	0.925	0.909	
	0.852			

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 calibrator is approximately 0.01 ng/mL.

Hook Effect

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

Specificity

This assay measures human C-peptide without any cross-reaction to Insulin.

Reproducibility and Precision

The intra-assay precision was validated by measuring 3 control samples with 16 replicate determinations. The inter-assay precision was validated by measuring 2 control levels in duplicate in 7 individual assays. The results are as follows:

Sample	Intra-Assay			Inter-Assay	
	1	2	3	1	2
Mean (ng/mL)	0.666	1.918	2.760	0.636	2.651
CV (%)	7.7	8.6	6.1	2.7	6.5

Linearity

Two EDTA plasma samples were collected and tested. The results are as follows:

Samples	Observed (ng/mL)	Recovery (%)
Sample A	0.959	-
50%	0.489	102.0
25%	0.281	117.2
12.5%	0.148	123.5
Sample B	1.855	-
50%	1.003	108.1
25%	0.505	108.9
12.5%	0.272	117.3

Two **serum** samples were collected, spiked with various amounts of C-peptide and tested. The results of C-peptide percent recovery value in ng/mL are as follows:

Dilution	Observed Value (ng/mL)	Recovery (%)
Neat A	0.844	-
1:2	0.416	98.6
1:4	0.214	101.4
1:8	0.106	100.5
Neat B	2.084	-
1:2	1.032	99.0
1:4	0.555	106.5
1:8	0.293	112.5

Spike Recovery

Two **EDTA plasma** samples and three assay calibrators (0.3, 1 and 3.5 ng/mL) were combined at equal volumes and tested. The results are as follows:

Dilution	Observed Value (ng/mL)	Recovery (%)
Neat A	1.851	-
Std-2: 0.3 ng/mL	0.968	90.0
Std-3: 1.0 ng/mL	1.227	86.1
Std-4: 3.5 ng/mL	2.348	87.8
Neat B	0.807	-
Std-2: 0.3 ng/mL	0.528	95.4
Std-3: 1.0 ng/mL	0.769	85.1
Std-4: 3.5 ng/mL	1.787	83.0

Two **serum** samples and three assay calibrators (0.3, 1 and 3.5 ng/mL) were combined at equal volumes and tested. The results are as follows:

Dilution	Observed Value (ng/mL)	Recovery (%)
Neat A	0.698	-
Std-2: 0.3 ng/mL	0.500	100.2
Std-3: 1.0 ng/mL	0.772	90.7
Std-4: 3.5 ng/mL	1.827	87.0
Neat B	4.344	-
Std-2: 0.3 ng/mL	2.064	88.9
Std-3: 1.0 ng/mL	2.801	104.9
Std-4: 3.5 ng/mL	3.398	86.6

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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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

This product is developed and manufactured by



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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD

In Vitro
Diagnostic
Device

RUO

For Research
Use Only

LOT

Lot Number

REF

Catalog Number



Read instructions
before use



Number of Tests



Store at



Use by



Keep away from
heat and direct
sun light



Manufacturer



Authorized
Representative
in Europe



European
Conformity

SHORT ASSAY PROCEDURE

1. Add **25 µL** of the calibrators, controls, and samples into the designated microwells.
2. Add **100 µL** of the HRP Conjugated Antibody into each microwell.
3. Mix, cover, and incubate at **room temperature (20-25 °C)** with **shaking for 60 minutes**.
4. Wash each well five times.
5. Add **100 µL** of substrate to each well.
6. Cover and incubate at **room temperature (20-25 °C)** for **20 minutes**.
7. Add **100 µL** of the stop solution to each well.
8. Read the absorbance at **450/620 or 450/650 nm**.