Quantikine® ELISA

Human C-Peptide Immunoassay

Catalog Number DICP00

For the quantitative determination of human C-Peptide concentrations in cell culture supernates, serum, plasma, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Insulin is a peptide hormone that is critical for glucose homeostasis. The mature Insulin peptide is derived from Proinsulin, which includes the Insulin A and B chains connected by a peptide fragment (C-peptide). Proinsulin is processed within the endoplasmic reticulum of pancreatic beta cells into equimolar ratios of mature Insulin and C-peptide. Human C-peptide is a 31 amino acid (aa) peptide that shares 58% and 61% aa sequence identity with the mouse and rat proteins, respectively. Initially, C-peptide was thought to be biologically inactive; however, studies now show that it facilitates microvascular circulation, nerve conduction, and renal function (1). Upon secretion into the portal circulation, a significant percentage of secreted Insulin is quickly metabolized by the liver. C-peptide is negligibly processed by the liver and can be used as marker of pancreatic beta cell function and endogenous Insulin secretion (2, 3).

C-peptide specifically and displaceably binds cell membranes with high affinity and activates an inhibitory G protein (Gi) coupled receptor (4-7). GPR146 may be a component of the C-peptide signaling complex (8). Administration of C-peptide can act in an anti-inflammatory capacity and can lead to improvements in microvascular blood flow and nerve function (4). C-peptide exerts vascular anti-inflammatory effects via the downregulation of endothelial cell surface adhesion molecules, the reduction of inflammatory cytokine production, and the protection of vascular smooth muscle cells from detrimental Insulin effects (9). Alternatively, high concentrations of C-peptide in Type II diabetics may contribute to vascular inflammation associated with atherosclerosis (10). C-peptide administration is associated with increased blood flow to multiple tissues and increases erythrocyte deformability in diabetic individuals (11, 12). It also activates and upregulates eNOS to increase nitric oxide (NO) synthesis, which is known to induce vasodilation (13, 14). Administration of C-peptide is associated with improvements in diabetic neuropathy and may act to increase nerve conduction and prevent neural dysfunction (15, 16). In addition, C-peptide may play a beneficial role in diabetic renal function (17).

Circulating and urinary ratios of Proinsulin, Insulin, C-peptide, and Creatinine are dysregulated in glucose homeostasis disorders (3). Hyperglycemia in Type I Diabetes Mellitus (DM) is caused by the failure of pancreatic beta cells to produce Insulin, while hyperglycemia in Type II DM is caused by peripheral Insulin resistance. Thus, as C-peptide can be used as a proxy for endogenous Insulin secretion, circulating C-peptide levels are reduced in Type I diabetics but are often elevated in Type II diabetics (18, 19). Further, as Type II DM progresses, Insulin production may decline leading to a decrease in urinary C-peptide:Creatinine ratios (20). C-peptide levels or C-peptide:Insulin ratios are also altered in individuals with renal, hepatic, and muscular disorders (21, 22). C-peptide levels are elevated in individuals with insulinoma, an insulin-secreting pancreatic tumor derived from beta cells (23, 24). It is important to note that as C-peptide levels reflect Insulin secretion, circulating C-peptide levels are affected by food intake and fasting.

The Quantikine® Human C-Peptide Immunoassay is a 2.5 hour solid-phase ELISA designed to measure human C-Peptide in cell culture supernates, serum, plasma, and urine. It contains synthetic human C-Peptide and has been shown to accurately quantitate the peptide. Results obtained using natural human C-Peptide showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human C-Peptide.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human C-Peptide has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any C-Peptide present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human C-Peptide is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of C-Peptide bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Samples, controls, and standards must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human C-Peptide Microplate	894589	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human C-Peptide.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human C-Peptide Conjugate	894590	21 mL of a monoclonal antibody specific for human C-Peptide conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human C-Peptide Standard	894591	Synthetic human C-Peptide (aa 3-33) in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-48	895911	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:10 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 250 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human C-Peptide Controls (optional; R&D Systems®, Catalog # QC108).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly icteric samples are not suitable for use in this assay.
Samples with abnormally high levels of Albumin interfere in this assay.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 125 µL of sample + 125 µL of Calibrator Diluent RD5-48 (diluted 1:10).*

Urine samples require a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD5-48 (diluted 1:10)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

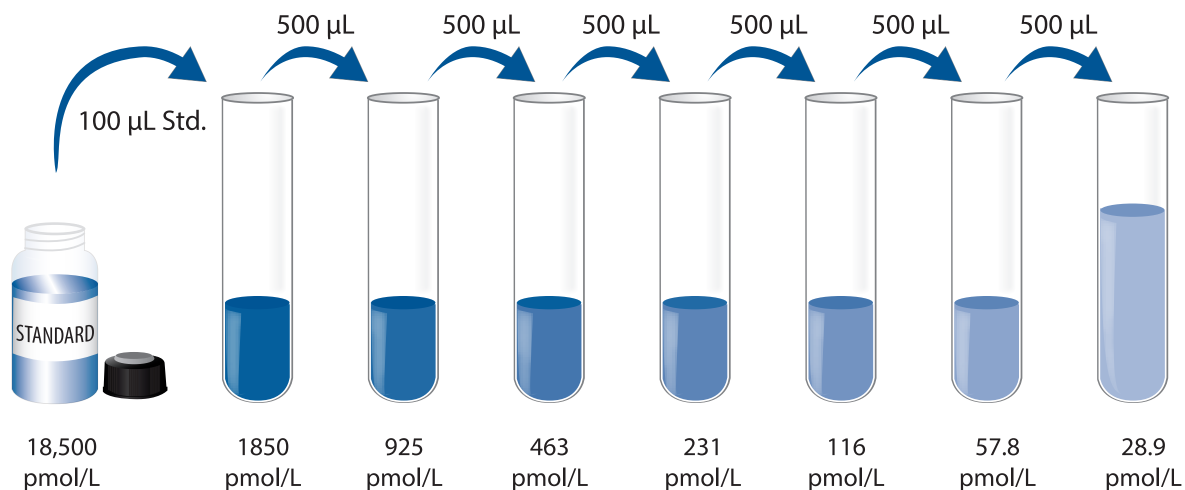
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-48 (diluted 1:10) - Add 20 mL of Calibrator Diluent RD5-48 to 180 mL of deionized or distilled water to prepare 200 mL of Calibrator Diluent RD5-48 (diluted 1:10).

Human C-Peptide Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human C-Peptide Standard with deionized or distilled water. This reconstitution produces a stock solution of 18,500 pmol/L. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-48 (diluted 1:10) into the 1850 pmol/L tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1850 pmol/L standard serves as the high standard. Calibrator Diluent RD5-48 (diluted 1:10) serves as the zero standard (0 pmol/L).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 100 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature.

   Note: *Samples, controls, and standards must be pipetted within 15 minutes.*
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human C-Peptide Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See the Sample Preparation section.

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CALCULATION OF RESULTS

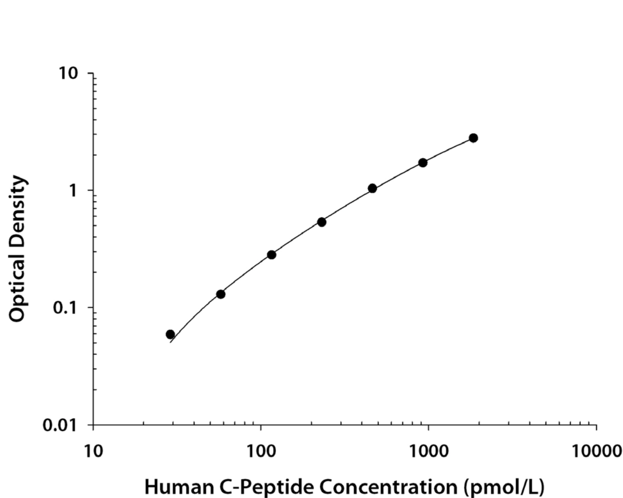
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human C-Peptide concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pmol/L)	O.D.	Average	Corrected
0	0.007 0.007	0.007	—
28.9	0.065 0.066	0.066	0.059
57.8	0.132 0.142	0.137	0.130
116	0.273 0.304	0.289	0.282
231	0.535 0.548	0.542	0.535
463	1.032 1.057	1.045	1.038
925	1.670 1.778	1.724	1.717
1850	2.674 2.911	2.793	2.786

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pmol/L)	129	436	845	127	437	861
Standard deviation	5.77	14.3	13.2	12.4	33.0	65.3
CV (%)	4.5	3.3	1.6	9.8	7.6	7.6

RECOVERY

The recovery of human C-Peptide spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	84-106%
Serum* (n=4)	103	96-109%
EDTA plasma* (n=4)	101	95-108%
Heparin plasma* (n=4)	102	90-115%
Urine* (n=4)	97	81-106%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human C-Peptide were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	99	103	106	104	101
	Range (%)	98-101	94-109	100-110	99-107	98-104
1:4	Average % of Expected	99	102	106	104	101
	Range (%)	95-103	95-106	101-110	101-107	97-104
1:8	Average % of Expected	102	100	102	101	100
	Range (%)	93-114	87-106	97-107	97-105	96-106
1:16	Average % of Expected	96	104	104	103	102
	Range (%)	92-100	94-113	100-107	100-106	98-104

*Samples were diluted prior to assay.

SENSITIVITY

Twenty-four assays were evaluated and the minimum detectable dose (MDD) of human C-Peptide ranged from 0.346-2.88 pmol/L. The mean MDD was 0.975 pmol/L.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against the NIBSC/WHO International Reference Reagent for C-Peptide 84/510.

The conversion factor for C-Peptide is 1 pmol = 3.0 pg/mL

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human C-Peptide in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pmol/L)	Range (pmol/L)	Standard Deviation (pmol/L)
Serum (n=35)*	700	330-1449	290
EDTA plasma (n=35)*	722	341-1566	300
Heparin plasma (n=35)*	675	340-1338	275
Urine (n=10)	7658	2369-27,680	7828

*Samples were from fasting individuals.

Cell Culture Supernates - Forty-one different cell lines and primary cell culture supernates were assayed for human C-Peptide. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and synthetic human C-Peptide.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human C-Peptide control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
IGF-I
IGF II
Insulin
Relaxin-1
Relaxin-2
Relaxin-3

Recombinant mouse:
IGF-I
IGF-II

Recombinant rat:
IGF-I

Recombinant human ProInsulin cross-reacts approximately 0.4% in this assay.

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