C-Peptide ELISA Kit

Catalog Number KA1259

96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

The C-Peptide ELISA is an enzyme immunoassay for the quantitative measurement of C-Peptide in serum, plasma and urine.

Background

Insulin is synthesized in the pancreatic beta cells as a 6000 MW component of an 86 amino acid polypeptide called proinsulin (1, 2, 3). Proinsulin is subsequently cleaved enzymatically, releasing insulin into the circulation along with a residual 3000 MW fragment called connection ("C") peptide, so-named because it connects A and B chains of insulin within the proinsulin molecule (1, 2, 3, 4). Human C-Peptide, a 31 amino acid residue peptide, has a molecular mass of approximately 3000 daltons. C-Peptide has no metabolic function. However, since C-Peptide and insulin are secreted in equimolar amounts, the immunoassay of C-Peptide permits the quantitation of insulin secretion (4, 5, 6). This is the reason for the clinical interest of serum and urinary determinations of C-Peptide. Moreover, C-Peptide measurement has several advantages over immunoassays of insulin.

The half-life of C-Peptide in the circulation is between two and five times longer than that of insulin (7). Therefore, C-Peptide levels are a more stable indicator of insulin secretion than the more rapidly changing levels of insulin. A very clear practical advantage of C-Peptide measurement arising from its relative metabolic inertness as compared to insulin is that C-Peptide levels in peripheral venous blood are about 5-6 times greater than insulin levels (3). Also, relative to an insulin assay, the C-Peptide assay's advantage is its ability to distinguish endogenous from injected insulin.

Thus, low C-Peptide levels are to be expected when insulin is diminished (as in insulin-dependent diabetes) or suppressed (as a normal response to exogenous insulin), whereas elevated C-Peptide levels may result from the increased β-cell activity observed in insulinomas (3, 6, 9).

C-Peptide has also been measured as an additional means for evaluating glucose tolerance and glibenclamide glucose tests (2, 3, 9, 10).

C-Peptide levels are in many ways a better measurement of endogenous insulin secretion than peripheral insulin levels. C-Peptide may be measured in either blood or urine (9). With improved sensitive C-Peptide immunoassays, it is now possible to measure C-Peptide values at extremely low levels. The clinical indications for C-Peptide measurement include diagnosis of insulinoma and differentation from factitious hypoglycemia, follow-up of pancreatectomy, and evaluation of viability of islet cell transplants (11, 12, 13).

Recently, these indications have been dramatically expanded to permit evaluation of insulin dependence in maturity onset diabetes mellitus.

Principle of the Assay

The C-Peptide ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The microtiter wells are coated with anti-mouse antibodies, which bind a monoclonal antibody directed towards a unique antigenic site on the C-Peptide molecule. Endogenous C-Peptide of the sample competes with a C-Peptide-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase conjugate is inversely proportional to the concentration of C-Peptide in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of C-Peptide in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Microtiterwells: 12 x 8 (break apart) strips; Wells coated with anti-mouse-antibody	96 wells
Standard (Standard 0-5): Lyophilized, Concentrations 0-16 ng/mL (see exact value on the QC-Datasheet). The standards are calibrated against WHO approved Reference material IRR C-Peptide, code 84/510. Contain non-mercury preservative.	0.75 mL x 6 vials
Sample Diluent: Ready to use, Contains non-mercury preservative	3 mL
Antiserum: Ready to use. Monoclonal mouse anti C-Peptide antibody contains non-mercury preservative.	7 mL
Enzyme Conjugate: Ready to use. Biotinylated C-Peptide contains non-mercury preservative.	14 mL
Enzyme Complex: Ready to use. Contains horseradish Peroxidase. Contains non-mercury preservative.	14 ml
Substrate Solution: Ready to use. TMB	14 mL
Stop Solution: Ready to use. Contains 1 N acidic solution. Avoid contact with the stop solution. It may cause skin irritations and burns.	14 mL
Wash Solution (40X concentrated)	30 mL

Storage Instruction

When stored at 2°C to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2°C to 8°C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Materials Required but Not Supplied

- ✓ A microtiter plate calibrated reader (450 ± 10 nm).
- ✓ Calibrated variable precision micropipettes.
- ✓ Absorbent paper.
- ✓ Distilled or deionized water.
- ✓ Timer.
- ✓ Semi logarithmic graph paper or software for data reduction

Precautions for Use

- Precautions

- ✓ All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- ✓ Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
- ✓ The microplate contains snap-off strips. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch and used in the frame provided.
- ✓ Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- ✓ Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- ✓ Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- ✓ Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- ✓ Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
- ✓ Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- ✓ Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- ✓ Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- ✓ Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- ✓ Do not use reagents beyond expiry date as shown on the kit labels.
- ✓ All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- ✓ Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- ✓ Avoid contact with Stop Solution containing 1 N acidic solution. It may cause skin irritation and burns.
- ✓ Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
- ✓ TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- ✓ Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

Assay Protocol

Reagent Preparation

Reagent Preparation: Bring all reagents and required number of strips to room temperature prior to use.

- Standards

  Reconstitute the lyophilized contents of the standard vial with 0.75 mL Aqua dest.

  Note: The reconstituted standards are stable for 3 days at 2°C to 8°C. For longer storage the reconstituted standards should be aliquoted and stored at -20°C.

- Wash Solution

  Add deionized water to the 40X concentrated Wash Solution.

  Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.

  The diluted Wash Solution is stable for 2 weeks at room temperature.

Sample Preparation

Serum or plasma (EDTA-, heparin- or citrate plasma) or urine can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Note: Samples containing sodium azide should not be used in the assay.

- Serum

✓ Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Donors receiving anticoagulant therapy may require increased clotting time.

✓ Specimens should be capped and may be stored for up to 24 hours at 2°C to 8°C prior to assaying.

✓ Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

- Plasma

✓ Whole blood should be collected into centrifuge tubes containing anti-coagulant and centrifuged immediately after collection.

✓ Specimens should be capped and may be stored for up to 24 hours at 2°C to 8°C prior to assaying.

✓ Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

- Urine

✓ The total volume of urine excreted during a 24 hour period should be collected and mixed in a single container.

- ✓ *Note: Specimens should be stored at 2-8°C during collection period and total volume collected should be recorded.*
- ✓ Aliquot a well-mixed sample to be used in the assay. Centrifuge sample to clear. Urine samples may be stored for up to 36 hours at 2-8°C prior to assaying.
- ✓ Specimens held for a longer time should be frozen only once at -20°C prior to assay.

- Sample Dilution

  If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Sample Diluent and reassayed as described in Assay Procedure.
  For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:
  a) dilution 1:10: 10 µL Serum + 90 µL Sample Diluent (mix thoroughly)
  b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Sample Diluent (mix thoroughly).

  Urine Samples
  Prior to use dilute urine samples 1:20 with Sample Diluent.

Assay Procedure

Note:
- ✓ *All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.*
- ✓ *Once the test has been started, all steps should be completed without interruption.*
- ✓ *Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.*
- ✓ *Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.*
- ✓ *As a general rule the enzymatic reaction is linearly proportional to time and temperature.*
- ✓ *Each run must include a standard curve.*

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 100 µL of each Standard, controls and samples with new disposable tips into appropriate wells.
3. Dispense 50 µL Antiserum into each well.
4. Dispense 100 µL Enzyme Conjugate into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for 60 minutes at room temperature with shaking (400-500 rpm).

6. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add 100 µL of Enzyme Complex to each well.
8. Incubate for 30 minutes at room temperature with shaking (400-500 rpm).
9. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
10. Add 100 µL of Substrate Solution to each well.
11. Incubate for 20 minutes at room temperature.
12. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well.
13. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader.
    It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

Data Analysis

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the protocol have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 16 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	1.82
Standard 1 (0.2 ng/mL)	1.64
Standard 2 (0.7 ng/mL)	1.46
Standard 3 (2.0 ng/mL)	1.02
Standard 4 (6.0 ng/mL)	0.47
Standard 5 (16 ng/mL)	0.21

Performance Characteristics

- Quality Control

✓ Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

✓ It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

✓ The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be

used for direct comparison of the results.

- ✓ It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
- ✓ Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid.
- ✓ In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
- ✓ After checking the above mentioned items without finding any error contact the distributor or Abnova directly.

- Expected Normal Values

  It is recommended that each laboratory should determine its own normal and abnormal values. In a study conducted with apparently normal healthy adults, using the kit the following values are observed:

Sample	n	Mean ± 2SD
Serum (Post 12-hour Fasting)	60	0.5 – 3.2 ng/mL
Urine		1 – 200 µg/day

- Assay Dynamic Range

  The range of the assay is between 0.06-16 ng/mL.

- Specificity of Antibodies (Cross Reactivity)

  The cross-reactivity of intact or split-Proinsulin is not significant.

- Sensitivity

  The analytical sensitivity of the ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Zero Standard (S0) and was found to be 0.064 ng/mL.

- Reproducibility

  Intra Assay

  The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	0.48	6.54
2	20	2.30	6.70
3	20	3.86	5.13

  Inter Assay

  The between assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	12	0.42	9.33

2	12	2.05	9.92
3	12	4.23	8.38

- Recovery

  Samples have been spiked by adding C-Peptide solutions with known concentrations in a 1:1 ratio. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Serum Sample	Endogenus C-Peptide ng/mL	Added C-Peptide ng/mL	Measured Conc. ng/mL	Expected Conc ng/mL	Recovery (%)
1	5.36	0.00	5.36		
		8.00	10.31	10.68	96.6
		3.00	5.57	5.68	98.0
		1.00	3.63	3.68	98.7
		0.35	3.08	3.03	101.8
2	9.70	0.00	9.70		
		8.00	12.49	12.85	97.2
		3.00	8.23	7.85	104.8
		1.00	5.15	5.85	87.9
		0.35	4.54	5.20	87.2
3	12.12	0.00	12.12		
		8.00	15.52	14.06	110.4
		3.00	9.72	9.06	107.3
		1.00	7.30	7.06	103.4
		0.35	5.65	6.41	88.1

Urine Sample	Endogenous C-Peptide (ng/mL)	Added Conc. 1:1 (v/v) (ng/mL)	Measured Conc. (ng/mL)	Expected Conc. (ng/mL)	Recovery (%)
1	2.1				
		8.0	10.9	10.1	107.9
		3.0	5.57	5.1	109.2
		1.0	2.6	2.62	99.2
2	1.01				
		8.0	9.2	9.01	102.1
		3.0	4.03	4.01	100.5
		1.0	2.2	2.01	109.5
3	2.5				
		8.0	10.1	10.5	96.2
		3.0	5.3	5.5	96.4
		1.0	3.8	3.5	108.6

- Linearity

Sample	Dilution	Measured Conc. ng/mL	Expected Conc. ng/mL	Recovery (%)
1	undil	6.10	6.10	
Serum	1:2	3.25	3.05	106.7
	1:4	1.61	1.52	105.3
	1:8	0.84	0.76	110.6
	1:16	0.41	0.38	107.6
2	undil	9.90	9.90	
Serum	1:2	5.59	4.95	112.8
	1:4	2.48	2.48	100.3
	1:8	1.29	1.24	104.0
	1:16	0.69	0.62	111.8
3	undil	13.25	13.25	
Serum	1:2	6.97	6.62	105.1
	1:4	3.22	3.31	97.1
	1:8	1.70	1.66	102.8
	1:16	0.85	0.83	103.1

Sample	Dilution	Measured Conc. ng/mL	Expected Conc. ng/mL	Recovery (%)
1	undil	8.7	8.7	
Urine	1:2	4.29	4.35	98.6
	1:4	2.01	2.18	92.4
	1:8	1.09	1.09	100.2
2	undil	9.2	9.2	
Urine	1:2	4.7	4.6	102.2
	1:4	2.25	2.3	97.8
	1:8	1.12	1.15	97.5
3	undil	13.9	13.9	
Urine	1:2	6.6	6.95	95.0
	1:4	3.3	3.48	95.0
	1:8	1.8	1.74	103.6

- Limitations of Use

  Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

✓ Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no

influence on the assay results.

- ✓ Drug Interferences

  Until today no substances (drugs) are known to us, which have an influence to the measurement of C-Peptide in a sample.

- ✓ High-Dose-Hook Effect

  No hook effect was observed in this test.

- Legal Aspects

  Reliability of Results

  The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

  The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

Resources

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Plate Layout

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