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ab178641

C-Peptide ELISA Kit

An immunoenzymatic assay for the quantitative measurement of C-Peptide in Human serum and plasma.

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

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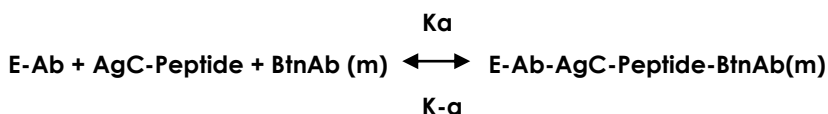
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1. Overview

Abcam's C-Peptide Human in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of C-Peptide in serum and plasma.

A 96-well plate has been precoated with Streptavidin. Samples, standards and the C-Peptide HRP and Biotin Conjugate are added to the wells. Biotinylated monoclonal and horseradish peroxidase (HRP) labelled antibodies are added and the reactants are mixed. The different types of antibodies used have high affinity and specificity and are directed against distinct and different epitopes of C-Peptide. Reaction between the various C-Peptide antibodies and native C-Peptide occurs in the microwells without competition or steric hindrance forming a soluble sandwich complex. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is directly proportional to the amount of C-Peptide in the sample and the intensity is measured at 450 nm.

The interaction is illustrated by the following equation:



BtnAb(m)	Biotinylated Monoclonal Antibody (Excess Quantity)
AgC-Peptide	Native Antigen (Variable Quantity)
E-Ab	enzyme labeled Antibody (Excess Quantity)
HRP-Ab(p)-AgC-Peptide-BtnAb(m)	Antigen-Antibodies Sandwich Complex
Ka	Rate Constant of Association
K-a	Rate Constant of Dissociation

Simultaneously, the complex is fixed to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

E-Ab-AgC-Peptide-BtnAb (m) + Streptavidin CW → Immobilized Complex

Streptavidin CW Streptavidin immobilized on well.

Immobilized Complex Antibodies-Antigen sandwich bound.

C-Peptide is the abbreviation for connecting peptide; it is a 31-amino acid peptide. C-Peptide of insulin is the C-terminal cleavage product produced during processing of the insulin pro-hormone to the mature insulin molecule. Proinsulin is cleaved when it is released from the pancreas into the blood - one C-Peptide for each insulin molecule. C-Peptide is devoid of any biological activity but appears to be necessary to maintain the structural integrity of Insulin. In-vitro determination of Insulin and C-Peptide level help in differential diagnosis of liver disease, acromegaly, Cushing syndrome, familial glucose intolerance, Insulinimia, renal failure, ingestion of accidental oral hypoglycaemic drugs or C-Peptide induced factitious hypoglycaemia.

Newly diagnosed diabetes patient often have their C-Peptide levels measured, to find if they have type 1 diabetes or type 2 diabetes. The pancreas of patients with type 1 diabetes is unable to produce insulin

and they will therefore usually have a decreased level of C-Peptide, while C-Peptide levels in type 2 patients is normal or higher than normal. Measuring C-Peptide in patients injecting insulin can help to determine how much of their own natural insulin these patients are still producing.

C-Peptide assays may be analytically more sensitive than insulin assays. Measurement of the C-Peptide may be useful in evaluating endogenous insulin secretion in a variety of clinical conditions. Insulin and C-Peptide are secreted into portal circulation in equimolar concentrations; fasting levels of C-Peptide are 5 – 10 fold higher than those of Insulin owing to the longer half-life of C-Peptide. The liver does not extract C-Peptide however; it is removed from the circulation by degradation in the kidneys with a fraction passing out unchanged in urine. Hence the urine C-Peptide levels correlate well with fasting C-Peptide levels in serum.

2. Protocol Summary

Prepare all reagents, samples and standards as instructed.



Add standards, control and samples into their respective wells. Add C-Peptide HRP and Biotin Conjugate to each well. Incubate at room temperature.



After washing, add TMB Substrate Solution to each well. Incubate at room temperature.



Add Stop Solution. Read at 450 nm.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website <https://www.abcam.com/en-us>.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Streptavidin Coated Microplate (12 x 8 wells)	96 wells	4°C
Stop Solution	15 mL	4°C
C-Peptide HRP and Biotin Conjugate	13 mL	4°C
TMB Substrate Solution	15 mL	4°C
50X Washing Solution	20 mL	4°C
C-Peptide Standard 0 – 0 ng/mL (Lyophilized)	1 vial	4°C
C-Peptide Standard 1 – 0.2 ng/mL (Lyophilized)	1 vial	4°C
C-Peptide Standard 2 – 1.0 ng/mL (Lyophilized)	1 vial	4°C
C-Peptide Standard 3 – 2.0 ng/mL (Lyophilized)	1 vial	4°C
C-Peptide Standard 4 – 5.0 ng/mL (Lyophilized)	1 vial	4°C
C-Peptide Standard 5 – 10.0 ng/mL (Lyophilized)	1 vial	4°C
Strip Holder	1 unit	4°C
Cover Foil	1 unit	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 μL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

8. Technical Hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Washing Solution

Prepare 1X Washing Solution by diluting 50X Washing Solution with deionized water. To make 500 mL 1X Washing Solution combine 10 mL 50X Washing Solution with 490 mL deionized water. Mix thoroughly and gently.

9.2 C-peptides Standard

The standards are lyophilized. Reconstitute each standard with 2 ml of distilled or deionized water.

Once reconstituted the standards are stable 7 days at 2-8°C.

In order to store for a longer period aliquot the reconstituted standards in vials and store at -20°C (stable for 6 months). Do not freeze thaw more than once.

All other solutions are supplied ready to use.

10. Sample Preparation

- Use Human serum or plasma samples with this assay. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. If the assay is performed within 5 days of sample collection, the specimen should be Use Human serum or plasma samples with this assay. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. If the assay is performed within 5 days of sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing.
- Samples with concentrations over 10 ng/mL should be diluted with C-Peptide Standard 0.
- Avoid repeated freezing and thawing.

11. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition.
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
- If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.
- Assay all standards, controls and samples in duplicate.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

12.3 Add 50 µL standards, control and samples into their respective wells. Add 100 µL C-Peptide HRP and Biotin Conjugate to each well. Leave a blank well for substrate blank.

12.4 Cover wells with the foil supplied in the kit and incubate for 2 hours at room temperature (22 - 28°C).

12.5 Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

ΔNote: Complete removal of liquid at each step is essential for good assay performance.

12.6 Add 100 µL TMB Substrate Solution into all wells.

12.7 Incubate for exactly 15 minutes at room temperature in the dark.

12.8 Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.

- 12.9** Measure the absorbance of the sample at 450 nm within 5 minutes of addition of the Stop Solution against a reference wavelength of 620-630 nm or against the blank.

13. Calculations

- Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).
- Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

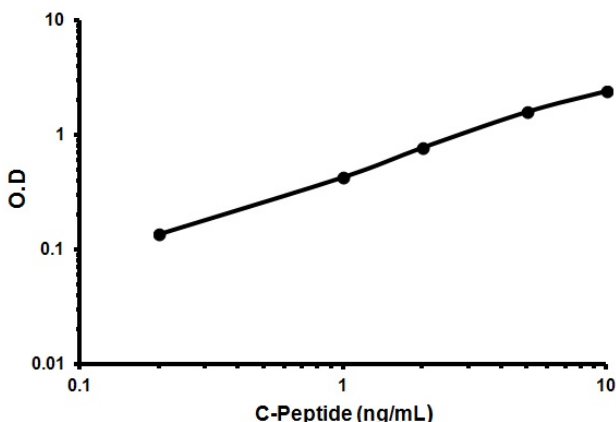


Figure 1. Example of C-peptide standard curve.

15. Typical Sample Values

REFERENCE VALUES –

C-Peptide values are consistently higher in plasma than in serum; thus, serum is preferred. Based on the clinical data gathered in concordance with the published literature the following guideline range has been assigned:

Adult (Normal) 0.7 – 1.9 ng/ml

SENSITIVITY –

The lowest detectable concentration of C-Peptide that can be distinguished from the standard 0 is 0.01 ng/mL at the 95 % confidence limit.

PRECISION –

	Intra-Assay	Inter-Assay
n=	16	20
%CV	≤ 6.2	≤ 10.0

16. Assay Specificity

The cross reactivity was evaluated by adding the interfering substances. The cross reactivity was calculated by deriving a ratio between the dose of interfering substance to the dose of C-Peptide needed to produce the same absorbance.

Cross Reagent	Conc. Tested	Obtained	Cross Reactivity
C-Peptide	---	---	100%
Insulin	10000 μIU/ml	N.D.	Not Detected
Pro-insulin	1000 ng/mL	N.D.	Not Detected

Please contact our Technical Support team for more information.

17.Troubleshooting

Problem	Cause	Solution
Low signal	Incubation time too short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. Notes

Technical Support

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