

DILUTION TEST: Linearity

Urine	Dilution factor	Measured conc. ng/ml	Expected Conc. ng/ml	Recovery %
1	Undiluted	3,83	8.7	-
	1:2	2,00	4.35	98.6
	1:4	0,92	2.18	92.4
	1:8	0,54	1.09	100.2
2	Undiluted	4,70	9.2	-
	1:2	2,39	4.6	102.2
	1:4	1,18	2.3	97.8
	1:8	0,57	1.15	97.5
3	Undiluted	7,33	13.9	-
	1:2	3,83	6.95	95.0
	1:4	1,78	3.48	95.0
	1:8	0,87	1.74	103.6

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. We recommend using BIO RAD Lypchocek Immunoassay Control Sera, which are also available from .

LIMITATION OF PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.
- Complete mixing of Conjugate with standard or sample (step 5) and of Stop Solution with Substrate Solution (step 14) is critical. Insufficient mixing will result in poor precision.

REFERENCES

- Ashby, J. and Frier, B.: Circulating C-Peptide: Measurement and Clinical Applications. *Annals of Clinical Biochemistry*. 18:125, 1981.
- Beischer, W.: Proinsulin and C-Peptide in Humans. *Hormones in Normal and Abnormal Human Tissues*. Volume 3K, Fotherby and Pal, S., ed. (Berlin: Walter DeGruyter). pp. 1-43, 1983
- Beyer, J., Krause V., Cordes V.: C-Peptide: Its Biogenesis, Structure, Determination and Clinical Significance. *Giornale Italiano di Chimica Clinica 4 Supp.* 9:22, 1979
- Bonger, A. and Garcia-Webb, P.: C-Peptide Measurement: Methods and Clinical Utility. *CRC Critical Reviews in Clinical Laboratory Sciences*. 19:297, 1984.
- Blix, P. Boddie-Wills, C., Landau, R., Rochman, H. Rubenstein, A.: Urinary C-Peptide: An Indicator of Beta-Cell Secretion under Different Metabolic Conditions. *Journal of Clinical Endocrinology and Metabolism*, 54:574, 1982.
- Rendell, M.: C-Peptide Levels as a Criterion in Treatment of Maturity-Onset Diabetes. *Journal of Clinical Endocrinology and Metabolism*. 57 (6): 1198, 1983
- Horwitz, D., et al.: Proinsulin, Insulin and C-Peptide concentrations in Human Portal and Peripheral Blood. *Journal of Clinical Investigation*. 55:1278, 1975

2008-12-18 (Mfg.:05-10-05)

**C-Peptide ELISA**

Catalog No. CP097D (96 Tests)

INTENDED USE

The Calbiotech, Inc. (CBI) C-Peptide ELISA Kit is used for quantitative determination of C-Peptide in serum, plasma and urine.

SUMMARY AND EXPLANATION

Human C-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. 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. 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. Also, relative to an insulin assay, the C-Peptide assay's advantage is its ability to distinguish endogenous from injected insulin. C-Peptide has also been measured as an additional means for evaluating glucose tolerance and glibenclamide glucose tests. 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. 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 differentiation from factitious hypoglycemia, follow-up of pancreatotomy, and evaluation of viability of islet cell transplants. Recently, these indications have been dramatically expanded to permit evaluation of insulin dependence in maturity onset diabetes mellitus.

PRINCIPLE OF THE TEST

The C-Peptide Elisa Kit is based on the competition principle and the microplate separation. An unknown amount of C-Peptide present in the sample and a fixed amount of C-Peptide Conjugate compete for the binding sites of a polyclonal C-Peptide antiserum coated onto the wells. In a second step an Enzyme Complex binds to C-Peptide Conjugate. The unbound Enzyme Complex is washed off. Having added the Substrate Solution, the concentration of C-Peptide in the samples is inversely proportional to the optical density measured.

MATERIALS PROVIDED	96 Tests
Microwells coated with anti-mouse -Ab	12x8x1
Standards (0-5) 6 vials	0.75 ml
Sample diluent	3 ml
Antiserum	7 ml
Enzyme Conjugate	14 ml
Enzyme Complex	14 ml
TMB Solution	14 ml
Stop Solution	14 ml
Wash Solution 40X	30 ml

MATERIALS NOT PROVIDED

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbance paper or paper towel
- Graph paper

STORAGE AND STABILITY

- Store the kit at 2 – 8° C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- Do not expose test reagents to heat, sun or strong light

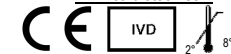
WARNINGS AND PRECAUTIONS


- Potential biohazardous materials:
The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level

Cat#: CP097D (96 Tests)

For Order and Inquiries, please contact

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2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984

2. This test kit is USA FDA exempt product.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. It is recommended that serum samples be run in duplicate.
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND PREPARATION

1. For Serum and Plasma:

The usual precautions for venipuncture should be observed. No special pretreatment of the sample is necessary. The specimen may be stored at 2-8° C for up to 24 hours, and should be frozen at -10° C or lower for longer periods. Do not use grossly hemolyzed or grossly lipemic specimens. Samples suspected to contain C-Peptide concentration higher than the upper limit of the Standard Curve are to be diluted with Specimen Diluent.

2. 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, store at -10°C or lower until ready for assay.
- Assay sample at a 1:20 dilution with the Specimen Diluent.

REAGENT PREPARATION

Standards:

Reconstitute the lyophilized standards with 1.0 ml distilled water. Allow them to remain undisturbed until completely dissolved, and then mix well by gentle inversion.

Wash Solution:

Add Aqua dest. to the 40X concentrated wash solution (contents: 30 ml) to a final volume of 1200 ml, if the complete plate is used at once. The diluted wash solution is stable for 8 weeks at 2-8° C.

Assay Procedure

All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the 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.
5. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
6. Incubate for 60 minutes at room temperature.
7. Briskly shake out the contents of the wells.
8. 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!
9. Add 100 µl of Enzyme Complex to each well.
10. Incubate for 30 minutes at room temperature.
11. Briskly shake out the contents of the wells.
12. Rinse the wells 3 times with diluted Wash Solution (400 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
13. Add 100 µl of Substrate Solution to each well.
14. Incubate for 20 minutes at room temperature.
15. Stop the enzymatic reaction by adding 100 µl of Stop Solution to each well.
16. Read the OD at 450±10 nm with a microtiter plate reader within 15 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

Any microwells reader capable of determining the absorbance at 450±10nm may be used. The C-Peptide value of each serum sample is obtained as follows:

1. Using linear-linear or semi log graph paper, construct a standard curve by plotting the average absorbance (Y) of each Reference Standard against its corresponding concentration (X) in ng/ml. For construction of the standard curve we recommend a four parameter logistic function.
2. Use the average absorbance of each serum sample to determine the corresponding C-Peptide value by simple interpolation from this standard curve, multiplying by the initial sample dilution.

EXPECTED VALUES

Normal range for serum, urine: It is recommended that each laboratory establish its own range of normal C-Peptide level. The normal range values observed with C-Peptide ELISA KIT with normal adult males and females are as follows:

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

Additionally, a glucose test was performed post 12-hour fasting with six normal adults. Serum was drawn after 12 hours of fasting. Participants were then administered 50-60 grams of glucose and samples again drawn after 30-45 minutes. C-Peptide levels increased after administration of glucose by 200-600 %.

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
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

Sensitivity: The lowest detectable level of C-Peptide that can be distinguished from the Zero Standard is 0.05 ng/ml.

Specificity: The cross-reactivity of intact or split-pro-insulin is clinically not significant.

Precision: Intra-assay

Inter-assay

Serum	n	Mean (ng/ml)	CV %	Inter-assay		
				n	<X> ± SD ng/ml	CV %
1	20	0.48	6.54	12	0.42	9.33
2	20	2.30	6.70	12	2.05	9.92
3	20	3.86	5.13	12	4.23	8.38

Accuracy -RECOVERY

Serum	Endogenous 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	98.7
		1.00	3.63	3.68	101.8
		0.35	3.08	3.03	
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	Endogenous C-Peptide ng/ml	Added C-Peptide 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