Monobind Inc.
Lake Forest, CA 92630, USA

AccuLite CLIA Microwells

C-Peptide Test System
Product Code: 2775-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Circulating C-Peptide Concentrations in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Diabetes is one of the leading causes of disability and death in the U.S. It affects an estimated 16 million Americans, and about one third do not know they have the disease. The causes of diabetes are not precisely known, but both genetic and environmental factors play a significant role. The disease is marked by deficiencies in the body's ability to produce and properly use insulin. The most common forms of diabetes are type 1, in which the body's ability to produce insulin is destroyed, and type 2, in which the body is resistant to insulin even though some amount of insulin may be produced.

In-vitro determination of insulin and *C-Peptide* levels help in the differential diagnosis of liver disease, acromegaly, Cushing's syndrome, familial glucose intolerance, insulinoma, renal failure, ingestion of accidental oral hypoglycemic drugs or insulin induced factitious hypoglycemia. Both insulin and *C-Peptide* are produced by enzymatic cleavage of proinsulin. Proinsulin is stored in the secretory granules of pancreatic β-cells and is split into a 31 amino acid connecting peptide (*C-Peptide;* MW 3600) and insulin (MW 6000). *C-Peptide* is devoid of any biological activity, but appears to be necessary to maintain the structural integrity of insulin. Although 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, urine *C-Peptide* levels correlate well with fasting *C-Peptide* levels in serum. The glucagon stimulated *C-Peptide* determination is often used for differential diagnosis of insulin-dependent from non-insulin-dependent diabetic patients.

3.0 PRINCIPLE

Chemiluminescence immunoassay (Type 3)
The essential reagents required for an chemiluminescence assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin (S.Avidin) coated on the well and exogenously added biotinylated monoclonal anti-C-Peptide antibody (Ab).

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen (Ag), a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(M)} + Ag_{C\text{-}Pep} + {}^{Btn}Ab_{(M)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(M)} - Ag_{C\text{-}Pep} - {}^{Btn}Ab_{(M)}$$

$^{Btn}Ab_{(M)}$ = Biotinylated Monoclonal Ab (Excess Quantity)
$Ag_{C\text{-}Pep}$ = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(M)}$ = Enzyme labeled Monoclonal Ab (Excess Quantity)
$^{Enz}Ab_{(M)} - Ag_{C\text{-}Pep} - {}^{Btn}Ab_{(M)}$ = Antigen-Antibodies complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(M)} - Ag_{C\text{-}Pep} - {}^{Btn}Ab_{(M)}$ + Streptavidin $_{CW}$ ⇒ Immobilize complex
Streptavidin$_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity, in the antibody-bound fraction, is directly proportional to the native antigen concentration. The enzyme activity is determined by reaction with a light emitting substrate. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS AND MATERIALS PROVIDED

Materials Provided:

A. C-Peptide Calibrators - 2ml/vial (Lyophilized) - Icons A-F
Six (6) vials of references for C-Peptide antigen at levels of 0(**A**), 0.2(**B**), 1.0(**C**), 2.0(**D**), 5.0(**E**), and 10.0(**F**) ng/ml. Reconstitute each vial with 2.0ml of distilled or deionized water. The reconstituted calibrators should be assayed immediately and can be stored for 8 hours at 2-8°C, then discarded. In order to store for a longer period of time, aliquot the reconstituted calibrators in cryo vials and store at -20°C for up to 30 days. Single use only. A preservative has been added.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRP 84/510.

B. C-Peptide Tracer Reagent – 13 ml/vial – Icon
One (1) vial containing enzyme labeled affinity purified monoclonal mouse antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Light Reaction Wells – 96 wells – Icon ⇓
One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C (see Reagent Preparation Section).

E. Signal Reagent A – 7ml/vial – Icon C[A]
One (1) vial containing luminol in buffer. Store at 2-8°C (see Reagent Preparation Section).

F. Signal Reagent B – 7ml/vial – Icon C[B]
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C (see Reagent Preparation Section).

G. Product Insert.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well microplate.

4.2 Required but not provided:
1. Pipette capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for signal reagent preparation.
4. Test tubes for dilution of signal reagent A and B
5. Microplate washer or a squeeze bottle (optional).
6. Microplate luminometer
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µL) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations

8.0 REAGENT PREPARATION AND STORAGE

1. **Wash Buffer**
   Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2 - 8°C.
   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). Discard the unused portion if not used within 36 hours after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with assay, bring all reagents, reference calibrators and controls to room temperature (20-27°C).

*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates wells for calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100ml (100µl) of the C-Peptide Tracer Reagent to each well. **It is very important to dispense all reagents close to the bottom of the microwell.**
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap.
5. Incubate for 60 minutes at room temperature
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat four (4) additional times.**
8. Add 0.100ml (100µl) of working signal reagent solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION
9. Incubate at room temperature for five (5) minutes in the dark.
10. Read the Relative Light Units (RLUs) using a 96 well microplate luminometer for 0.2 – 1.0 seconds per well. **The results should be read within thirty (30) minutes of adding the signal solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of C-Peptide in unknown specimens.
1. Record the RLUs obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLUs for each duplicate serum reference versus the corresponding C-Peptide concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of C-Peptide for an unknown, locate the average RLUs for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (12864) of the patient intersects the calibration curve at 1.18ng/ml C-Peptide concentration (See Figure 1)*.

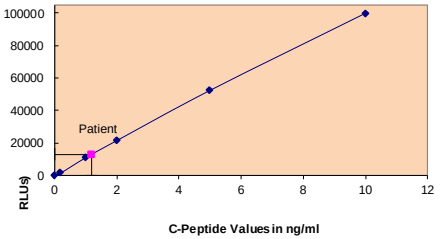
Note 1: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion eliminates differences cause by efficiency of the various instruments that can be used to measure light output

EXAMPLE 1

Sample I.D.	Well Number	RLU	Mean RLU	Value (ng/ml)
Cal A	A1	163	161	0
	B1	160		
Cal B	C1	1402	1432	0.2
	D1	1462		
Cal C	E1	10416	10837	1
	F1	11257		
Cal D	G1	20703	21370	2
	H1	22038		
Cal E	A2	54430	52387	5
	B2	50387		
Cal F	C2	100672	100000	10
	D2	99328		
Ctrl 1	E2	15212	15610	1.43
	F2	16008		
Ctrl 2	G2	61273	61198	5.84
	H2	61122		
Patient	A3	12617	12864	1.18
	B3	13110		

Figure 1



11.0 QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve (80%; 50% & 20% intercepts) should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available upon request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Patient samples with C-Peptide concentrations above 10 ng/ml may be diluted with the zero calibrator and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis – as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedures have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin.Chem.* 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED VALUES

C-Peptide values are consistently higher in plasma than in serum; thus, serum is preferred. Compared with fasting values in non-obese non-diabetic individuals, C-Peptide levels are higher in obese non-diabetic subjects and lower in trained athletes.

Each laboratory is advised to establish its own ranges for normal and abnormal populations. These ranges are always dependent upon locale, population, laboratory, technique and specificity of the method.

Based on the clinical data gathered by Monobind in concordance with the published literature the following ranges have been assigned. **These ranges should be used as guidelines only.**

Adult (Normal)	**0.7 – 1.9 ng/ml**

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the C-Peptide AccuLite® CLIA Test System were determined by analyses on two different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Pool 1	20	1.49	0.12	7.7%
Pool 2	20	5.97	0.35	5.8%
Pool 3	20	12.00	0.15	1.3%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Pool 1	20	1.30	0.11	8.7%
Pool 2	20	6.10	0.60	9.8%
Pool 3	20	13.16	1.25	9.5%

*As measured in ten experiments in duplicate over seven days.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.012 ng/ml.

14.3 Accuracy

The C-Peptide AccuLite® CLIA Test System was compared with a reference method. Biological specimens from population (symptomatic and asymptomatic) were used. The values ranged from 0.2 ng/ml – 11.8ng/ml. The total number of such specimens was 133. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Method (y)	1.068	y = 0.2079 + 0.8036(x)	0.962
Reference (x)	1.066		

Only slight amounts of bias between the C-Peptide AccuLite® CLIA Test System and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the C-Peptide AccuLite® CLIA Test System to selected substances was evaluated by adding the interfering substance(s) to a serum matrix at the following concentration(s). The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of C-Peptide needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
C-Peptide	1.000	-
Proinsulin	0.120	100 ng/ml
Insulin	non-detectable	1.0 mIU/ml
Glucagon	non-detectable	150 ng/ml

14.5 High Dose Hook-Effect

The test will not be affected by C-Peptide concentrations up to 100 ng/ml in serum. However, samples expected to be over 10 ng/ml should be diluted 1:10 and 1:50 in normal pooled human serum and the normal pool assayed along side to obtain a base value. The base value and dilution factor should be taken into account to get the corrected concentration of C-Peptide in the sample.

15.0 REFERENCES

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Revision: 6 **Date: 2019-Jul-16** **DCO: 1353**
MP2775 Product Code: 2775-300

Size		96(A)	192(B)
Reagent (fill)	A)	2ml set	2ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)

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IVD 2° 8° CE
EC REP CEpartner4U, Esdoornlaan 13 3951 DBMaarn, The Neatherlands www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

- IVD — In Vitro - Diagnostic Medical Device
- 2° 8° — Temperature Limitation Storage Condition (2-8°C)
- Consult Instructions for Use
- REF — Catalogue Number
- Σ — Contains Sufficient Test for Σ
- LOT — Batch Code
- Used By (Expiration Day)
- Date of Manufacturer
- Manufacturer
- EC REP — Authorized Rep in European Country
- CE — European Conformity