



## C-peptide ELISA

For the quantitative determination of C-peptide in serum or plasma

For "*In Vitro* Diagnostic" use within the United States of America.  
This product is for "Research Use Only" outside of the United States of America.

Catalog Number:	80-CPTHU-E01.1
Size:	96 wells
Version:	v2.3: April 20, 2010

### **ALPCO Diagnostics**

26G Keewaydin Drive • Salem, NH 03079  
Phone: (800) 592-5726 • Fax: (603) 898-6854  
[www.alpco.com](http://www.alpco.com) • Email: [web@alpco.com](mailto:web@alpco.com)

**INTENDED USE**

The ALPCO C-peptide ELISA is designed for the quantitative determination of C-peptide in serum or plasma.

**PRINCIPLE OF THE ASSAY**

The ALPCO C-peptide ELISA is a sandwich type immunoassay. The 96-well microplate is coated with a monoclonal antibody specific for C-peptide. The C-peptide Standards, Diabetes Controls, and samples are added to the microplate wells with Assay Buffer. After incubation on an orbital microplate shaker at 700-900 rpm, the wells are washed with Wash Buffer and blotted dry. The Conjugate (horseradish peroxidase enzyme-labeled monoclonal antibody) is then added, and the microplate is incubated a second time on an orbital shaker at 700-900 rpm, washed, and blotted dry. TMB Substrate is added, and the microplate is incubated a third time on an orbital microplate shaker at 700-900 rpm. Once the third incubation period is complete, Stop Solution is added, and the optical density (OD) is measured by spectrophotometer at 450 nm with a reference wavelength of 620 nm. The intensity of the color generated is directly proportional to the amount of C-peptide in the sample.

**MATERIALS SUPPLIED****Catalog Number: 80-CPTHU-E01.1**

<b>Components</b>	<b>Content</b>	<b>Quantity</b>	<b>Preparation</b>
C-peptide Microplate (coated with monoclonal anti-C-peptide antibody)	1 microplate	12 x 8 well strips	Ready to use
Zero Standard (0 pmol/L )	1 vial	5 ml	Lyophilized
Standards (A-E) (20, 100, 300, 1000, 3000 pmol/L)*	5 vials	1 ml/vial	Lyophilized
Diabetes Controls (Levels 1 and 2)	2 vials	0.6 ml/vial	Lyophilized
Assay Buffer	1 vial	6 ml	Ready to use
Conjugate Stock	1 vial	1.2 ml	Concentrate
Conjugate Buffer	1 bottle	12 ml	Ready to use
Wash Buffer (21X)	1 bottle	40 ml	Concentrate
TMB Substrate	1 bottle	12 ml	Ready to use
Stop Solution	1 bottle	12 ml	Ready to use
Plate Sealers	3	-	Ready to use

\*The concentrations listed above are approximate; please refer to the Certificate of Analysis enclosed with each kit for the lot specific concentrations.

### **MATERIALS REQUIRED BUT NOT SUPPLIED**

- Precision pipettes with disposable tips capable of dispensing 25 µl, 50 µl, and 100 µl
- Repeating or multi-channel pipette capable of dispensing 50 µl and 100 µl
- Volumetric container and pipettes for reagent preparation
- Deionized or distilled water
- Microplate washer or wash bottle
- Orbital microplate shaker capable of 700-900 rpm
- Microplate reader with 450 and 620-650 nm filter

### **PRECAUTIONS**

1. The human blood products incorporated into this kit have been tested for the presence of HIV (Human Immunodeficiency virus), HBV (Hepatitis B virus), and HCV (Human Hepatitis C virus). Test methods for these viruses do not guarantee the absence of virus; therefore all reagents should be treated as potentially infectious. Handling and disposal should be in accordance with all appropriate national and local regulations for the handling of potentially biohazardous materials.
2. All materials derived from animal sources are BSE negative. However, all materials should be kept from ruminating animals.
3. Avoid direct contact with skin.
4. This product is not for internal use.
5. Avoid eating, drinking, or smoking when using this kit.
6. Do not pipette any components by mouth.
7. Components from this kit should not be mixed with components of different lot numbers.
8. Do not use components beyond the expiry date.
9. Variations to the test procedure are not recommended and may influence the test results.

### **SAMPLE HANDLING**

Serum or plasma (heparin or EDTA) samples are appropriate for use in this assay. No dilution or treatment of the sample is required. If a sample value is greater than the highest standard, the sample should be diluted in Zero Standard, and the analysis should be repeated.

Samples can be stored at 2-8°C for 24 hours prior to analysis. Storage at < -20°C for longer periods is recommended. Avoid repeated freeze/thaw cycles.

### **REAGENT PREPARATION AND STORAGE CONDITIONS**

- The kit should be stored at 2-8°C. The kit is stable until the expiry date on the box label.
- All reagents must reach room temperature prior to preparation and subsequent use in the assay.

**Conjugate Stock** is an 11X concentrate. Dilute Conjugate Stock with 10 parts Conjugate Buffer. For example, to prepare Working Strength Conjugate for one microplate, dilute 1.2 ml of Conjugate Stock with 12 ml of Conjugate Buffer. Working Strength Conjugate is stable for 30 days at 2-8°C.

**Diabetes Controls (Levels 1 and 2)** are provided in a lyophilized form. Reconstitute each control with 0.6 ml of deionized water. Close the vial with the rubber stopper and cap, then gently swirl the vial and allow it to stand for 30 minutes prior to use. The contents of the vial should be in solution with no visible particulates. The reconstituted controls are stable for 1 day stored at 2-8°C. For longer term storage the controls should be stored in aliquots at < -20°C for up to 6 months. Avoid repeated freeze/thaw cycles.

**Wash Buffer (21X)** is diluted with 20 parts deionized water. For example, to prepare Working Strength Wash Buffer, dilute 20 ml of Wash Buffer (21X) with 400 ml of deionized water. Working Strength Wash Buffer is stable for 30 days at room temperature (18-25°C).

**Standards (A-E) and the Zero Standard** are provided in a lyophilized form. Reconstitute Standards (A-E) with 1 ml of deionized water each. Reconstitute the Zero Standard with 5 ml of deionized water. If multiple assays are to be performed, the standards should be stored in aliquots at -20°C. Avoid repeated freeze/thaw cycles.

### **QUALITY CONTROL**

It is recommended that the Diabetes Controls provided with the ALPCO C-Peptide ELISA be included in every assay. The concentration range of the controls is provided on the Certificate of Analysis enclosed with each kit; however, it is recommended that each laboratory establishes its own acceptable range.

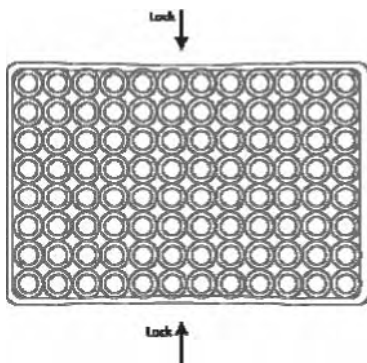
### **ASSAY PROCEDURE**

**Bring all reagents to room temperature prior to use.** Briefly vortex all reagents before use. A standard curve must be performed with each assay and with each microplate if more than one microplate is run at a time. All standards, controls, and samples should be run in duplicate.

1. Designate enough microplate strips for the standards, controls, and desired number of samples. The remaining strips should be stored in the tightly sealed foil pouch containing the desiccant and stored at 2-8°C.
2. **Pipette 25 µl** of each reconstituted standard, reconstituted control, and sample into the corresponding wells. (See *Reagent Preparation*.)
3. **Pipette 50 µl** of Assay Buffer into each well.
4. Cover microplate with a plate sealer and **incubate for 1 hour**, shaking at 700-900 rpm on an orbital microplate shaker at room temperature (18-25°C).
5. **Wash the microplate 6 times** with Working Strength Wash Buffer (see *Reagent Preparation*) with a microplate washer. Alternatively, use a wash bottle to fill the wells, and then discard the liquid, inverting and firmly tapping the microplate on absorbent paper towels between washes. After the final wash with either the microplate washer or wash bottle, remove any residual Wash Buffer and bubbles from the wells by inverting and firmly tapping the microplate on absorbent paper towels (See *Microplate Locking Diagram* below).
6. **Pipette 100 µl** of Working Strength Conjugate (see *Reagent Preparation*) into each well.
7. Cover microplate with a plate sealer and **incubate for 1 hour**, shaking at 700-900 rpm on an orbital microplate shaker at room temperature (18-25°C).
8. **Wash the microplate 6 times** with Working Strength Wash Buffer (as in Step 5).

9. **Pipette 100  $\mu$ l** of TMB Substrate into each well.
10. Cover microplate with a plate sealer and **incubate for 15 minutes** on an orbital microplate shaker (700-900 rpm) at room temperature (18-25°C).
11. **Pipette 100  $\mu$ l** of Stop Solution into each well. Gently shake the wells to stop the reaction. Remove bubbles before reading with a microplate reader.
12. Place the microplate in a microplate reader capable of reading the absorbance at 450 nm with a reference wavelength of 620-650 nm. The microplate should be analyzed within 30 minutes following the addition of the Stop Solution.

### ***Microplate Locking Diagram***



When tapping the microplate onto the absorbent paper grasp at lock points indicated by the arrows, locking the microplate strips into place.

This locking feature allows for tapping with greater force than with traditional microplates.

### ***CALCULATION OF RESULTS***

A calibration curve is constructed from the C-peptide Standards. It is preferable to use a software program to calculate the standard curve and to determine the concentration of the samples. The preferred calculation method is cubic spline. The Zero Standard should be used as a blank with its average value subtracted from each well. Plot the standard curve using a log/log scale.

**Manual Calculation:** The Zero Standard should be used as a blank with its average value subtracted from each well. The standard concentrations are plotted on the X-axis and the absorbance values are plotted on the Y-axis using log/log paper. The sample concentrations are determined by plotting the absorbance of each unknown sample against the standard curve. The corresponding value on the X-axis is the concentration of the unknown sample.

### ***EXPECTED VALUES***

The ALPCO C-Peptide ELISA is calibrated to the WHO C-Peptide 1st IRP 84/5 10. It is recommended that each laboratory establishes its own normal range for its patient population.

Conversion for Human C-peptide to grams: 1 pmol/L of Human C-peptide = 3.6 pg/ml

## PERFORMANCE CHARACTERISTICS

### Sensitivity:

The analytical sensitivity was determined by calculating the mean  $\pm$  2 standard deviations for 20 replicates of the Zero Standard. The sensitivity of the assay is 2.95 pmol/L.

### Precision: Within run (intra-assay) variation

The within run precision is expressed as the percentage coefficient of variation (CV%). This was determined based on the mean and standard deviation of 20-32 replicates of a sample run in a single assay. The table below shows the results of 3 samples that span the range of the assay.

	Sample 1	Sample 2	Sample 3
mean	346.33 pmol/L	1387.05 pmol/L	2788.11 pmol/L
std. dev.	15.59 pmol/L	58.98 pmol/L	79.92 pmol/L
CV%	4.50	4.25	2.87
n	20	32	24

### Precision: Between run (inter-assay) variation

The between run precision is expressed as the percentage coefficient of variation (CV%). This was determined based on the mean and standard deviation across 11 assays of duplicate measurements of a single sample. The table below shows the results of 3 samples that span the range of the assay.

	Sample 1	Sample 2	Sample 3
Mean	337.22 pmol/L	1031.77 pmol/L	2243.00 pmol/L
std. dev.	28.48 pmol/L	89.90 pmol/L	148.32 pmol/L
CV%	8.4	8.7	6.6
N	11	11	11

### Linearity:

The linearity of the assay was determined by preparing dilutions of a sample with a high C-peptide concentration with the Zero Standard. The expected values were compared to the obtained values to determine a percent recovery. The range of recovery was 88-109%.

### Spike and Recovery:

The spike and recovery for the assay was determined by adding various known amounts of C-peptide to a sample. This spiked sample was evaluated in the assay and the measured concentration was compared to the expected concentration (endogenous + spiked). The range of recovery was 82-111% with an average of 99%.

**Specificity:**

The table below indicates the analyte and the percent cross-reactivity observed in the assay.

Analyte	% Cross-reactivity
Human insulin	3.0
Human proinsulin (intact)	<0.01
Humalog	0.24
NovoLog	ND*
Humulin R	ND
Porcine insulin	ND
Porcine C-peptide	ND
Rat C-peptide I	ND
Rat C-peptide II	ND
Mouse C-peptide I	ND
Mouse C-peptide II	ND
Rat proinsulin I	ND
Rat proinsulin II	ND
Mouse proinsulin I	ND
Mouse proinsulin II	ND

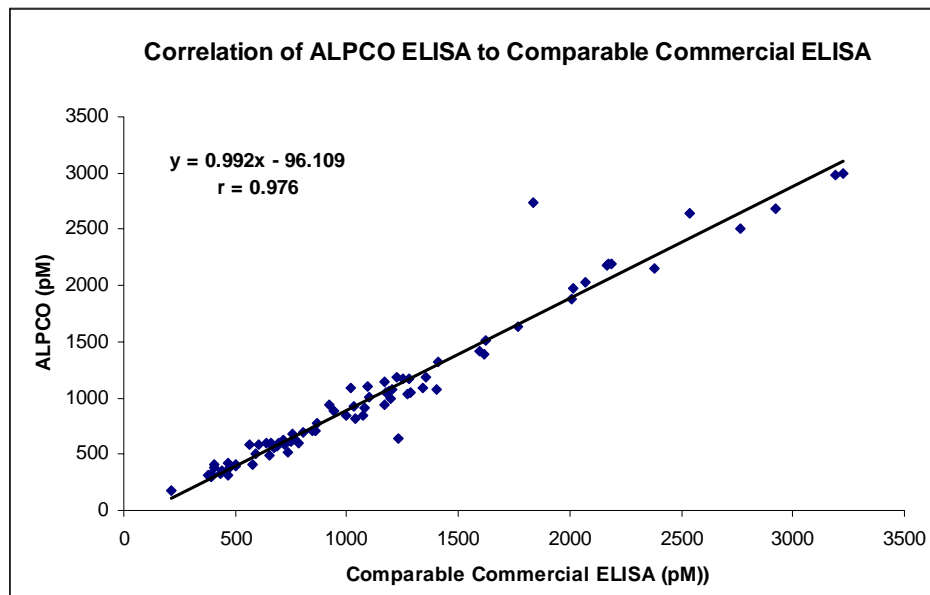
\*ND = Not detected.

**Hook Effect:**

No high dose hook effect was observed with C-peptide concentrations up to 48,852 pmol/L.

**Accuracy:**

The ALPCO C-peptide ELISA was evaluated for accuracy by comparing its performance to that of another commercially available C-peptide ELISA. The graph below shows the results of 80 samples evaluated using both methods. The correlation coefficient between the two methods is  $r = 0.976$ .



## C-peptide ELISA Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

### 80-CPTHU-E01.1 Short Protocol:

