Rat C-peptide ELISA

For the quantitative determination of C-peptide in rat serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 80-CPTRT-E01

Size: 96 wells

Version: May 26, 2016

26-G Keewaydin Drive, Salem, NH 03079 | P: (800) 592-5726 | F: (603) 898-6854 | ts@alpco.com | www.alpco.com

INTENDED USE

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

PRINCIPLE OF THE ASSAY

The ALPCO Rat C-peptide ELISA is a sandwich type immunoassay. The 96-well microplate is coated with a monoclonal antibody specific for C-peptide. The standards, controls, and samples are added to the microplate wells with the conjugate. The microplate is then incubated at room temperature on a microplate shaker at 700-900 rpm. After the first incubation is complete, the wells are washed with Wash Buffer and blotted dry. TMB Substrate is added, and the microplate is incubated a second time at room temperature on a microplate shaker at 700-900 rpm. Once the second incubation is complete, Stop Solution is added, and the optical density (OD) is measured by a spectrophotometer at 450 nm. The intensity of the color generated is directly proportional to the amount of C-peptide in the sample.

MATERIALS SUPPLIED

80-CPTRT-E01		
Component	**Quantity**	**Preparation**
C-peptide Microplate, 96 wells	12 x 8 strips	Ready to use
Zero Standard	5 ml	Ready to use
Standards (A-E)*	5 vials	Lyophilized*
High and Low Controls*	1 vial each	Lyophilized*
Conjugate Stock	1.2 ml	11X
Conjugate Buffer	12 ml	Ready to use
Wash Buffer Concentrate	40 ml	21X
TMB Substrate	12 ml	Ready to use
Stop Solution	12 ml	Ready to use
Plate Sealers	3	Ready to use

*Please refer to the Certificate of Analysis enclosed with each kit for more information.

MATERIALS REQUIRED

- Precision pipettes for dispensing up to 100 µl (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 100 µl
- Volumetric containers and pipettes for reagent preparation
- Distilled or deionized water for reagent preparation
- Microplate washer or wash bottle
- Microplate shaker capable of 700-900 rpm
- Microplate reader with 450 nm filter
- Vortex for sample preparation

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 (Hepatitis C virus). Test methods for these viruses do not guarantee the absence of a virus; therefore, all reagents should be treated as potentially infections. 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 product.
6. Do not pipette any reagents by mouth.
7. Reagents from this kit are lot-specific and must not be substituted.
8. Do not use reagents beyond the expiration date.
9. Variations to the test procedure are not recommended and may influence the test results.

STORAGE CONDITIONS

The kit should be stored at 2-8°C. The kit is stable until the expiration date on the box label.

SAMPLE HANDLING

Serum samples are appropriate for use in this assay. No dilution or treatment of the sample is required. However, if a sample has a greater concentration of C-peptide than the highest standard, the sample should be diluted in Zero Standard and the analysis should be repeated.

It is recommended to 1) thoroughly vortex each sample before use and 2) perform pipetting actions without pausing.

Store C-peptide samples in aliquots at ≤ -20°C. Samples can undergo up to 4 freeze/thaw cycles.

REAGENT PREPARATION

All reagents must be equilibrated to room temperature prior to preparation and subsequent use in the assay.

Conjugate Stock is to be diluted with 10 parts Conjugate Buffer. For example, to prepare enough Working Strength Conjugate for one complete microplate, dilute 1.0 ml of Conjugate Stock (11X) with 10 ml of Conjugate Buffer. Prepare an appropriate volume of Working Strength Conjugate immediately before use in the assay.

Wash Buffer Concentrate is to be diluted with 20 parts distilled water. For example, to prepare Working Strength Wash Buffer, dilute 20 ml of Wash Buffer Concentrate (21X) with 400 ml of deionized water. Working Strength Wash Buffer is stable for 4 weeks at room temperature (18-25°C).

Controls (High and Low) and Standards (A-E) are provided in a lyophilized form. Please refer to the Certificate of Analysis provided with each kit for the appropriate volume of deionized water for reconstitution. Close each vial with the rubber stopper and cap, 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.

- Store the reconstituted controls and standards in aliquots at ≤ -20°C for up to 6 months. Avoid repeated freeze/thaw cycles.

QUALITY CONTROL

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

ASSAY PROCEDURE

All reagents and microplate strips should be equilibrated to room temperature (18-25°C) prior to use. Gently mix all reagents before use. A standard curve must be performed with each assay run and with each microplate if more than one is used at a time. All standards, controls, and samples should be run in duplicate.

1. The microplate should be equilibrated to room temperature prior to opening the foil pouch. Designate enough microplate strips for duplicate determinations of the standards, controls, and samples. The remaining microplate strips should be stored at 2-8°C in the tightly sealed foil pouch containing the desiccant.
2. **Pipette 10 µl** of each standard, control, and sample into their respective wells. See *Reagent Preparation* and *Certificate of Analysis* for reconstitution instructions.
3. **Pipette 100 µl** of Working Strength Conjugate (see *Reagent Preparation*) into each well.
4. Cover microplate with a plate sealer and **incubate for 2 hours ± 15 minutes** at room temperature, shaking at 700-900 rpm on a microplate shaker.
5. Decant the contents of the wells and **wash the microplate 3 times** with 350 µl of Working Strength Wash Buffer per well (see *Reagent Preparation*) using a microplate washer. Alternatively, fill the wells with Working Strength Wash Buffer using a wash bottle (do not use a multichannel pipette). Discard the liquid, invert, and firmly tap the microplate on absorbent paper towels between washes. After the final wash, (automated or manual), remove any residual Wash Buffer and bubbles from the wells by inverting and firmly tapping the microplate on absorbent paper towels.
6. **Pipette 100 µl** of TMB Substrate into each well.
7. Cover microplate with a plate sealer and **incubate for 15 minutes ± 2 minutes** at room temperature, shaking at 700-900 rpm on a microplate shaker.
8. **Pipette 100 µl** of Stop Solution into each well and gently shake the microplate to mix the contents. Remove any bubbles before proceeding with the next step.
9. Place the microplate in a microplate reader capable of reading the absorbance at 450 nm. The microplate should be analyzed immediately after the addition of the Stop Solution, and no longer than 30 minutes after.

CALCULATION OF RESULTS

Construct a standard curve from the standards. Plot the zero standard as part of the curve. It is recommended to use a software program to calculate the standard curve and to determine the concentration of the samples.

The Rat C-peptide ELISA is a ligand binding assay, with responses exhibiting a sigmoidal relationship to the analyte concentration. Currently accepted reference models for such curves use a 5 parameter logistic (pl) fit, as this model optimizes the accuracy and precision across a

greater range. Although cubic spline and other models are acceptable methods, they generally show less intra-assay accuracy and precision at the low and high ends of the range.

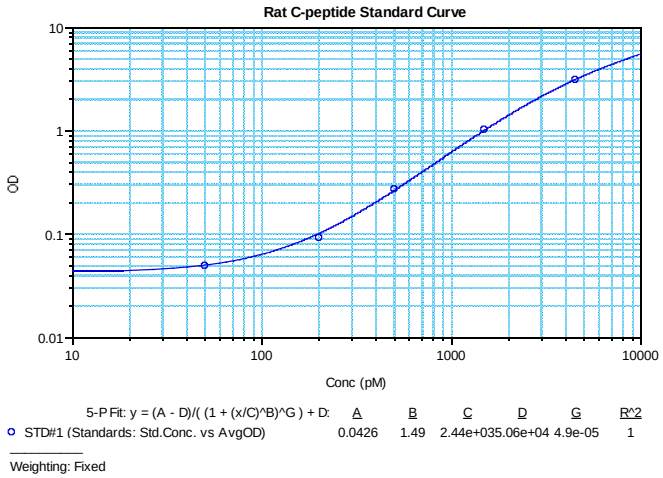
In the example below, a 5 pl curve fit was used to maximize the accuracy and precision of samples with low concentrations. However, the accuracy and precision of all models are limited at the lowest and highest ends of the detectable range due to the influence of individual laboratory conditions. As a result, caution should always be used when interpreting results where the analyte response becomes non-linear.[1]

Extrapolating sample concentration values outside the range of the standard concentration values is not recommended.

TYPICAL STANDARD CURVE

The following results are provided for demonstration purposes only and cannot be used instead of data obtained with the assay. A standard curve must be performed with each assay run and plate tested.

Standard	Conc. (pM)	OD
Zero	0	0.048
A	50	0.050
B	200	0.093
C	500	0.272
D	1500	1.023
E	4500	3.142



PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity was determined by calculating the mean ± 3 standard deviations for 40 replicates of the Zero Standard. The sensitivity of the assay is 10.8 pM.

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 replicates of a sample run in a single assay. The table below shows the results of 4 samples that span the range of the assay.

	Sample 1	Sample 2	Sample 3	Sample 4
Mean (pM)	2165.9	929.0	553.2	420.8
Std. Dev. (pM)	77.5	28.8	23.1	15.7
CV %	3.6	3.1	4.2	3.7
n	20	20	20	20

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 20 replicates of a single sample. The table below shows the results of 4 samples that span the range of the assay.

	Sample 1	Sample 2	Sample 3	Sample 4
Mean (pM)	1288.5	966.1	673.3	363.0
Std. Dev. (pM)	81.7	55.5	49.5	24.9
CV %	6.3	5.7	7.4	6.9
n	20	20	20	20

Linearity
The linearity of the assay was determined by preparing dilutions of a sample with high C-peptide concentrations with the Zero Standard. The expected values were compared to the obtained values to determine a percent recovery. The average recovery was 108%.

	Observed (pM)	Expected (pM)	% Recovery
Sample 1	4725	-	-
80%	4353	3780	115
60%	2717	2835	96
40%	1875	1890	99
20%	861	945	91
Sample 2	1036	-	-
80%	934	829	113
60%	738	621	119
40%	480	414	116
20%	241	207	116
Sample 3	900	-	-
80%	721	720	100
60%	522	540	97
40%	306	360	85
20%	127	180	70
Sample 4	2196	-	-
80%	1914	1757	109
60%	1612	1318	122
40%	1195	879	136
20%	649	439	148

Spike and Recovery
The spike and recovery of 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). Across 3 samples, the range of recovery was 92 – 102% with an average of 98%.

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

Analyte	% Cross-reactivity*
Rat C-peptide I	100
Rat C-peptide II	128.4
Mouse C-peptide I	83.8
Mouse C-peptide II	117.9
Porcine C-peptide	ND
Human C-peptide	ND
Rat proinsulin I	0.7
Rat proinsulin II	0.8
Mouse proinsulin I	1
Mouse proinsulin II	2.1
Porcine proinsulin	ND
Bovine proinsulin	ND
Human proinsulin	ND
Human insulin	ND

*ND = not detected.

Hook Effect
No high dose hook effect was observed with rat C-peptide concentrations up to 120,000 pM.

REFERENCES
1. Finlay JWA, Dillard RF. Appropriate Calibration Curve Fitting in Ligand Binding Assays. *AAPS Journal*. 2007; 9(2): E260-E267.

SHORT ASSAY PROTOCOL