Catalog #90055

V.6

Crystal Chem
High Performance Assays

Rat C-Peptide ELISA Kit Instructions

For the quantitative determination of c-peptide in rat serum, plasma, and fluid

Catalog #90055
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Rat C-Peptide ELISA Kit is for the quantitative determination of c-peptide in rat serum, plasma, and fluid. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

C-peptide is formed from pro-insulin and co-secreted with insulin. Measuring the amount of c-peptide is useful as an index of insulin secretion. The Rat C-Peptide ELISA kit is a simple, precise, and sensitive ELISA sandwich assay for rat c-peptide.

C. Principle of the Assay

The Rat C-Peptide ELISA kit is an ELISA sandwich assay for rat c-peptide. Rat c-peptide in the sample is first bound to the rabbit anti-c-peptide antibody coated on the microplate well. POD conjugated anti-c-peptide antibody is then bound to the complex immobilized on the microplate. After addition of the substrate solution, the concentration of rat c-peptide is interpolated from a standard curve based on the absorbance measured.

D. Kit Storage

1. Upon receipt of the Rat C-Peptide ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
A	Antibody-coated Microplate (One pack contains 6 x 8 wells, ie. 48 wells)	2 packs
B	Standard, Lyophilized	2 vials (2.56 ng/vial)
C	Anti-C-Peptide Enzyme Conjugate Solution	1 bottle (13 mL)
D	Enzyme Substrate (TMB) Solution	1 bottle (13 mL)
E	Enzyme Reaction Stop Solution (1 N Sulfuric Acid)	1 bottle (13 mL)
F	Sample Diluent	1 bottle (30 mL)
G	Wash Buffer Stock Solution (20X Concentrate)	1 bottle (50 mL)
	Frame for affixing the microplate well module	1 piece
	Plastic microplate cover	1 piece

E.2. Materials to be supplied by user

Micropipettes and disposable tips
Volumetric flasks
Distilled or deionized water
Polypropylene microtubes
Test tube racks
Vortex mixer
Aspirator for washing procedure
Microplate shaker (optional)
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents, including the Enzyme Substrate Solution and the Enzyme Reaction Stop Solution, should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes. In case of contact with eyes or skin, flush immediately with water and contact a medical professional.
2. Do not allow the Enzyme Substrate Solution to contact any metal.
3. Do not use the reagents after the expiration date.

G. Maximizing Kit Performance

1. Given the small sample volumes required (5 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. The wash procedure should be done thoroughly in order to minimize background readings.
4. Each calibrator and sample should be assayed in duplicate.
5. The same sequence of pipetting and other operations should be maintained in all procedures.
6. Do not mix reagents that have different lot numbers.

H. Sample Collection

Plasma: Collect blood into a tube containing an anticoagulant such as heparin (final concentration: 1 unit/mL), EDTA (final concentration: 0.1%), or sodium citrate (final concentration: 0.76%), and centrifuge at 4°C for 20 min at 2,000 x g.

Serum: Collect blood, allow to clot, and centrifuge at 4°C for 20 min at 2,000 x g.

Please note to avoid hemolysis during preparation. Do not use turbid serum or plasma samples. Turbid serum or plasma should be centrifuged to produce a clear solution. Samples which need to be diluted must be diluted with the Sample Diluent (marked "F").

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Remove the microplate from the foil pouch after the pouch has been equilibrated to room temperature.
   Note: *The microplate must be used the same day as the pouch is opened.*

2. Standard stock solution
   Reconstitute the "Standard, Lyophilized" (marked "B") by careful addition of 200 µL of Sample Diluent (marked "F") to the vial. Invert the vial gently until the contents are completely dissolved. This stock solution contains 12.8 ng/mL of c-peptide.
3. Anti-c-peptide enzyme conjugate
   Provided as ready to use.
4. Enzyme substrate solution
   Provided as ready to use. Once the bottle is opened, the enzyme substrate solution is stable for one week at 2-8°C.
   Note: *Avoid exposure of the enzyme substrate solution to light.*
5. Enzyme reaction stop solution (1 N sulfuric acid)
   Provided as ready to use.
6. Sample Diluent
   Provided as ready to use. Once the bottle is opened, the sample diluent is stable for one week at 2-8°C.
7. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water. Wash buffer is stable for one week at 2-8°C after dilution.

I.2. Preparation of working standards

1. Pipette 100 µL of Sample Diluent and 100 µL of the reconstituted standard (12.8 ng/mL) into a polypropylene microtube labeled 6.4 ng/mL, and mix thoroughly.
2. Dispense 100 µL of Sample Diluent into six polypropylene microtubes labeled 3.2, 1.6, 0.8, 0.4, 0.2, and 0.1 ng/mL,
3. Dispense 100 µL of the 6.4 ng/mL standard into the 3.2 ng/mL microtube, and mix thoroughly.
4. Dispense 100 µL of the 3.2 ng/mL standard into the 1.6 ng/mL microtube, and mix thoroughly.
5. Dispense 100 µL of the 1.6 ng/mL standard into the 0.8 ng/mL microtube, and mix thoroughly.
6. Repeat this dilution scheme using the remaining microtubes.
7. Dispense 100 µL of Sample Diluent into one polypropylene microtube labeled 0 ng/mL. You should now have working standards of 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1, and 0 ng/mL.

TABLE 2 Preparation of working standards

	Standard concentration (ng/mL)							
	6.4	3.2	1.6	0.8	0.4	0.2	0.1	0
SSS*(µL)	100							
SD**(µL)	100	100	100	100	100	100	100	100
		100	100	100	100	100	100	
Total (µL)	200	200	200	200	200	200	200	100

SSS*: Standard stock solution (12.8 ng/mL)
SD**: Sample Diluent

I.3. Assay procedure

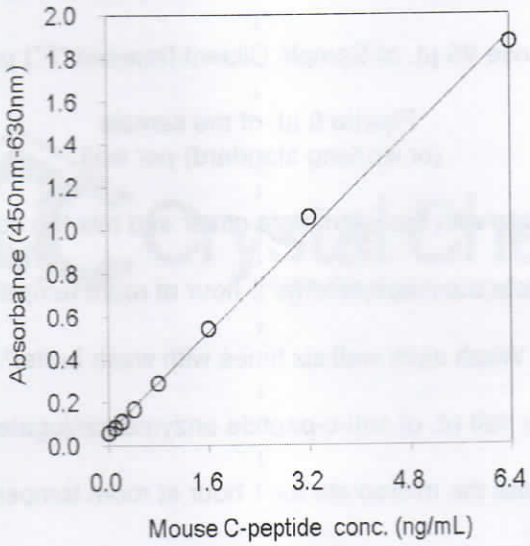
Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. Remove the antibody-coated microplate modules from the sealed foil pouch after the pouch has been equilibrated to room temperature. Affix the microplates to the supporting frame.
2. In each well, add 95 µL of Sample Diluent.
3. In each well, add 5 µL of sample or working standards.
   Note: *Each sample and working standard should be assayed in duplicate.*
4. Cover the microplate with the plastic microplate cover and mix the solution in each well for 10 seconds (shake the microplate on level table with hand or with microplate shaker).
5. Incubate the plate for 1 hour at room temperature.
6. Aspirate well contents and wash six times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of Anti C-Peptide Enzyme Conjugate in each well.
8. Cover the microplate with the plastic cover and incubate the plate for 1 hour at room temperature.
9. Aspirate well contents and wash six times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
10. Immediately dispense 100 µL per well of Enzyme Substrate Solution and react for 30 minutes at room temperature. During the enzyme reaction, avoid exposing the microplate to light.
    Note: *Do not cover the plate with aluminum foil.*
11. Stop the reaction by adding 100 µL of Enzyme Reaction Stop Solution.
12. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.4. Determining the c-peptide concentration

1. Determine the mean absorbance for each set of duplicate standards or samples.
   Note: *If individual absorbance values differ from the mean by greater than 20%, performing the assay again is recommended. The mean absorbance of the 0 ng/mL standard should be less than 0.1.*
2. Using linear graph paper, construct the standard curve by plotting the mean absorbance value for each standard on the Y axis versus the corresponding standard concentration on the X axis. Figure 1 is an example of a typical standard curve generated by the ELISA assay.
   Note: *A standard curve should be plotted every time the assay is performed.*
3. Rat c-peptide concentrations in the samples are interpolated using the standard curve and mean absorbance values for each sample. The c-peptide concentration is expressed in ng/mL. The unit of measure can be converted to pM by multiplying the obtained concentration in ng/mL by 320.3.
   Note: *Samples with high rat c-peptide concentrations (ie. fall above 6.4 ng/mL) should be further diluted with the sample diluent and rerun.*

FIGURE 1 A typical standard curve (linear fit)



J. Performance characteristics

J.1. Assay range

The Rat C-Peptide ELISA Kit has an assay range from 0.1 – 6.4 ng/mL.

J.2. Precision

The assay has a intra- and inter-assay precision of CV ≤ 10%.

J.3. Recovery

When rat c-peptide was spiked in a 5 µL rat serum sample, the recovery was 100% ± 20%.

K. Appendix

K.1. Summary of reagent preparation

TABLE 3 Summary of reagent preparation

Reagent	Preparation Procedure
A: Antibody-coated Microplate	Ready to use
B: Standard, Lyophilized	Dilute with 200µL of sample diluent
C: Anti-C-Peptide Enzyme Conjugate Solution	Ready to use
D: Enzyme Substrate (TMB) Solution	Ready to use
E: Enzyme Reaction Stop Solution (1N Sulfuric Acid)	Ready to use
F: Sample Diluent	Ready to use
G: Wash Buffer Stock Solution (20X Concentrate)	Bring contents of the bottle to 1L with distilled/deionized water

K.2. Summary of ELISA Assay

Affix the Antibody-coated Microplate (marked "A") to the frame.
↓
Dispense 95 µL of Sample Diluent (marked "F") per well.
↓
Pipette 5 µL of the sample
(or working standard) per well.
↓
Cover the microplate with the microplate cover and mix the solution in each well.
↓
Incubate the microplate for 1 hour at room temperature.
↓
Wash each well six times with wash buffer*.
↓
Dispense 100 µL of anti-c-peptide enzyme conjugate per well.
↓
Incubate the microplate for 1 hour at room temperature.
↓
Wash each well six times with wash buffer*.
↓
Dispense 100 µL of Enzyme Substrate Solution
(marked "D") per well.
↓
Cover the microplate with the plate sealer and incubate microplate for 30 min at room temperature while avoiding exposure to light.
↓
Stop the enzyme reaction by adding 100 µL of Enzyme
Reaction Stop Solution (marked "E") per well.
↓
Measure A450 and subtract A630 values within 30 min.
↓
Calculate C-peptide concentrations using the standard curve.

* Each well should be washed with 300 µL of wash buffer. Aspirate the wells completely so all excess solution is removed.

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