COSMO BIO CO., LTD.
Inspiration for Life Science

Rat C-Peptide EIA

Cat. No. YII-YK010-EX

FOR LABORATORY USE ONLY

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Contents

- Please read all the package insert carefully before beginning the assay -

YII-YK010-EX Rat C-Peptide EIA

Ⅰ. Introduction

This enzyme immunoassay (EIA) kit is a stable and convenient assay system for rat C-peptide in its plasma, serum, urine and culture supernatant

The processing of proinsulin, which occurs within the B cell, yields insulin and C-peptide and insulin and C-peptide are secreted in equimolar quantities into blood circulation. Therefore, the measurement of C-peptide in blood reflects the concentration of insulin and also provides valuable information to evaluate the pancreatic B cell function.

The EIA kit is using synthetic rat C-Peptide I as standard and biotinylated rat C-Peptide I as labeled antigen. The kit contains specific polyclonal antibody (C-peptide antibody) recognized to the amino acid sequence in the C-terminal side region which are common between rat C-Peptide I and II.

YK010 Rat C-Peptide EIA Kit	Contents
▼ The assay kit can measure C-Peptide in the range of 1.56-50 ng/mL ▼ The assay completed within 5.5 hours ▼ With one assay kit, 41samples can be measured in duplicate ▼ Test sample: Culture supernatant, plasma, serum and urine Sample volume: 50 µL ▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately. ▼ Precision and reproducibility Intra-assay CV (%) 3.38 - 8.83 Inter-assay CV (%) 5.56 - 8.41 ▼ Stability and Storage Store all of the components at 2-8°C. 24 months from the date of manufacturing. The expiry date is described on the label of kit.	1) Antibody coated plate 2) C-Peptide standard 3) Labeled antigen 4) C-Peptide antibody 5) SA-HRP solution 6) Substrate buffer 7) OPD tablet 8) Stopping solution 9) Buffer solution 10) Washing solution (concentrated) 11) Adhesive foil

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of rat C-Peptide in its plasma, serum, urine and culture supernatant samples. The kit is characterized for sensitive quantification, high specificity and no influences with other components in samples. Rat C-Peptide standard is highly purified synthetic product (purity: higher than 98%).

< Specificity >

The EIA kit has high specificity to rat C-Peptide and shows no cross reactivity to human and other animal species.

< Test Principle >

This EIA kit for determination of rat C-Peptide in plasma, serum, urine and culture supernatant samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to rat C-Peptide and biotin- avidin affinity system. The 96-wells plate is coated with goat anti rabbit IgG and C-Peptide standard or samples, labeled antigen and anti rat C-Peptide antibody are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP labeled streptoavidin (SA-HRP) be added to form HRP labeled streptoavidin-biotinylated rat C-Peptide-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of rat C-Peptide is calculated.

Ⅲ. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	MTP[*1]	1 plate (96 wells)	Goat Anti rabbit IgG
2. C-Peptide standard	lyophilized	1 vial	Synthetic rat C-Peptide I (50ng)
3. Labeled antigen	lyophilized	1 vial	Biotinylated rat C-Peptide I
4. C-Peptide antibody	liquid	1 bottle (12 mL)	Rabbit anti rat C-Peptide
5. SA-HRP solution	liquid	1 bottle (12 mL)	HRP labeled streptoavidin
6. Substrate buffer	liquid	1 bottle (24 mL)	0.015% Hydrogen Peroxide
7. OPD tablet	tablet	2 tablets	o-Phenylenediamine dihydrochloride
8. Stopping solution	liquid	1 bottle (12 mL)	2N H_2SO_4
9. Buffer solution	liquid	1 bottle (30 mL)	Phosphate buffer
10. Washing solution (Concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
11. Adhesive foil		3 sheets	

MTP[*1]..... Microtiter plate

Ⅳ. Method

< Equipment required >

1) Photometer for microtiter plate (Plate reader), which can read extinction 2.5 at 490 nm

2) Microtiter plate shaker

3) Washing device for microtiter plate and dispenser with aspiration system

4) Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips

5) Test tubes for preparation of standard solution

6) Graduated cylinder (1,000 mL)

7) Distilled water or deionized water

< Preparatory work >

1) Preparation of standard solution:
Reconstitute the C-Peptide standard (lyophilized rat C-Peptide I, 50ng/vial) with 1mL of buffer solution, which affords 50ng/mL standard solution. The 0.5ml of the reconstituted standard solution is diluted with 0.5 mL of buffer solution that yields 25ng/mL standard solution. The 0.5mL of 25 ng/mL standard solution is diluted with 0.5 mL of the buffer solution that makes 12.5ng/mL standard solution. Repeat the dilution to make each standard solution of 6.25, 3.12, 1.56 ng/mL. Buffer solution is used as 0 ng/mL.

2) Preparation of labeled antigen:
Reconstitute labeled antigen with 8mL of buffer solution.

3) Preparation of substrate solution:
Resolve OPD tablet with 11 mL of substrate buffer. It should be prepared immediately before use.

4) Preparation of washing solution:
Dilute 50 mL of washing solution (concentrated) to 1000 mL with distilled or deionized water.

5) Other reagents are ready for use.

< Procedure >

1. Bring all the reagents and samples return to room temperature before beginning the test.

2. Fill 50µL of buffer solution into wells first, then introduce 50µL each of standard solutions (0, 1.56, 3.12, 6.25, 12.5, 25, 50 ng/mL) or samples, then add 50µL of labeled antigen and finally introduce 100µL of C-Peptide antibody into the wells.

3. Cover the plate with adhesive foil and incubate it at room temperature (20-30°C) for 3 hours. During the incubation, the plate should be shake with a microtiter plate shaker.

4. Take off the adhesive foil, aspirate the solution in the wells and wash the wells three times with approximately 0.35 mL/well of washing solution.

5. Pipette 100µL of SA-HRP solution into the wells.

6. Cover the plate with adhesive foil and incubate it at room temperature (20-30°C) for 2 hours. During the incubation, the plate should be shake with a microtiter plate shaker.

7. Take off the adhesive foil, aspirate and wash the wells three times with approximately 0.35 mL/well of washing solution.

8. Resolve OPD tablet with 11 mL of substrate buffer. It should be prepared immediately before use. Add 100µL of substrate solution into the wells cover the plate with adhesive foil and incubate it for 10 minutes at room temperature.

9. Add 100µL of stopping solution into the wells to stop color reaction.

10. Read the optical absorbance of the wells at 490 nm. Calculate mean absorbance values of wells containing standards and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the standard curve to read C-Peptide concentrations in samples from the corresponding absorbance values.

Ⅴ. Notes

1. Samples must be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -20°C. Avoid repeated freezing and thawing of samples.

2. C-Peptide standard, labeled antigen, substrate solution should be prepared immediately before use. This kit can be used divisdedly in strips of the plate. In such case, the rest of reconstituted reagents (C-Peptide standard and Labeled antigen) should be stored below –30°C
.
3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2-8°C.

4. As pipetting operations may affect the precision of the assay, pipette precisely standard solutions or samples into each well of plate. Using clean test tubes or vessels in assay and use a new tip for each sample to avoid cross contamination.

5. When sample value exceeds 50 ng/mL, it needs to be diluted with buffer solution to proper concentration.

6. During incubation except color reaction, the test plate should be shake gently by microtiter plate shaker to promote immunoreaction.

7. During continuous shaking of test plate, the plate shaker may be heated up. It is recommended to place styrene foam or plywood between the plate and the shaker.

8. Perform all the determination in duplicate.

9. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

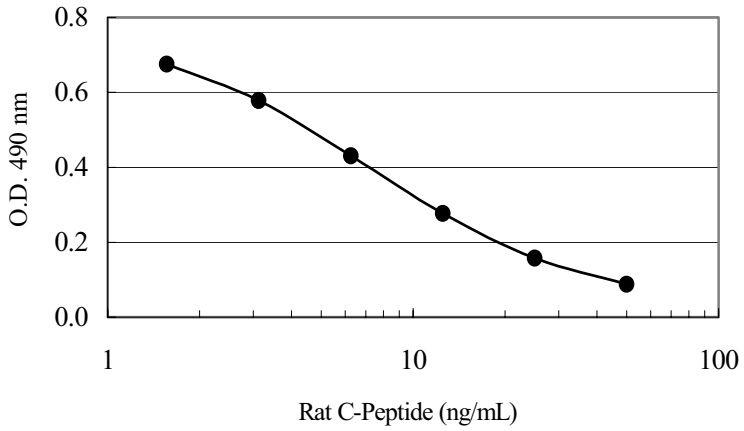
10. To quantitate accurately always run a standard curve when testing samples.

11. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

12. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

Ⅵ. Performance Characteristics

Typical standard curve



Analytical recovery

Rat C-Peptide added ng/mL	Observed ng/mL	Expected ng/mL	Recovery (%)
0.0	5.70		
1.0	6.26	6.13	102.20
5.0	10.20	10.10	100.60
25.0	32.20	30.10	106.90

Precision and reproducibility

- Intra-assay CV (%) 3.38 - 8.83
- Inter-assay CV (%) 5.56 - 8.41

Assay range

1.56 – 50 ng/mL

Ⅶ. Stability and Storage

< Storage > Store all of the components at 2-8°C.

< Shelf life > 24 months from the date of manufacturing
The expiry date is described on the label of kit.

< Package > For 96 tests per one kit including standards

Ⅷ. References

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Manufactured by Yanaihara Institute Inc.

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