Rat C-Peptide EIA Kit

Cat. No. YII-YK010-EX

FOR LABORATORY USE ONLY

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– Please resd all the package insert carefully before beginning the assay –

YK010 **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 and urine.
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 prepared by using synthetic rat C-Peptide I as standard antigen and biotinylated rat C-Peptide I as labeled antigen. The kit contains specific polyclonal antibody recognized to the amino acid sequence in the C-terminal side region which are common between rat C-Peptide I and II.

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of rat C-Peptide in its plasma, serum & urine samples. It has a lot of advantage to perform the assay, such as good quantification, no influence with other body fluid factors or physiogical active substances and needlessness of sample pretreatment. Rat C-Peptide standard is highly purified synthetic product (purity: higher than 98%) and biotinylated peptide is stable because N –biotinylglycylglycyl Rat C-Peptide I is used for it.

< 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 and urine samples is based on a competitive enzyme immunoassay using combination with 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, biotinylated rat C-Peptide and anti rat C-Peptide antibody are added to the wells for competitive immunoreaction. After rinsing out excess rat C-Peptide, HRP labeled streptoavidins are added to bind to the antigen-antibody complex so that HRP labeled streptoavidin - biotinylated rat C-Peptide – antibody complexs are formed on the surface on the wells. Finally, excess HRP labeled streptoavidins are rinsed out and HRP enzyme activity is determined 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)	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 hydrochloride
8. Stopping solution	liquid	1 bottle (12 mL)	2N-H_2SO_4
9. Buffer solution	liquid	1 bottle (35 mL)	Phosphate buffer
10. Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
11. Adhesive foil		3 sheets	

MTP*1..... Microtiration plate

Ⅳ. Method

< Equipment required >

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

2) Rotator for microtitration plate

3) Washing device for microtitration plate and dispenser for approximately 0.3 mL 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 50 ng/mL standard solution. 0.5ml of the reconstituted standard solution is diluted with 0.5 mL of buffer solution, that yields 25 ng/mL standard solution. 0.5mL of 25 ng/mL standard solution is diluted with 0.5 mL of the buffer solution, that makes 12.5 ng/mL standard solution. Repeat the dilution to make each standard 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. Warm up the reagents and samples to room temperature before beginning the test.

2. Fill 50μL of buffered 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 rotated with a plate rotator.

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 rotated with a plate rotator.

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

8. 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 reaction.

10. Read the optical absorbance of the wells at 490nm.

11. Calculate mean absorbance values of wells containing standards and plot a standard curve on semilogarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values.).

12. Use the standard curve to read C-Peptide concentrations in samples from the corresponding absorbance values.

Ⅴ. Notes

1. Plasma samples must be used as soon as possible after collection. If the samples are to be tested at a later time, they should be divided into test tubes in small amount and frozen at or below – 30°C. Avoid repeated freezing and thawing of plasma samples.

2. C-Peptide standard, labeled antigen, substrate solution should be prepared immediately before use in assay using clean test tubes or vessels. Diluted washing solution is stable for 6 months at 2 to 8°C.

3. During storage of washing solution (concentrated) at 2 to 8°C, precipitates may be observed, however they will be dissolved when diluted.

4. As pipetting operations may affect with the precision of the assay, pipette precisely standard solutions or samples into each well of plate. And use 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 within the assay range.

6. During incubation except color reaction, the test plate should be rotated gently by plate rotator to promote immunoreaction.

7. During continuous rotation of test plate, the plate rotator may be heated up. It is recommended to place styrene form or plywood between the plate and the rotator.

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

9. Perform all the determination in duplicate.

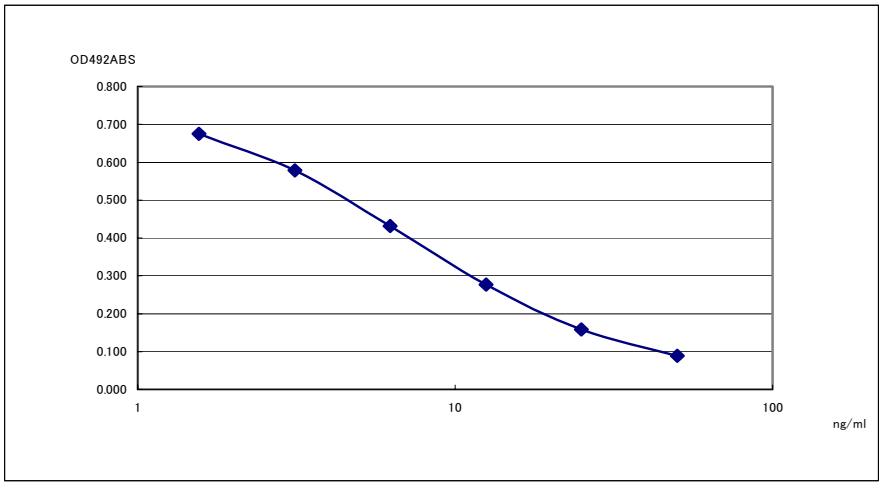
10. To quantitate accurately, always run a standard curve when testing samples.

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	5.7		
1.0	6.26	6.13	102.2
5.0	10.2	10.1	100.6
25.0	32.2	30.1	106.9

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 to 8°C.

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

< Package > For 96 tests per 1 kit including standards

Ⅷ. References

1. Markussen, J. and Sundby, F. (1972): Eur. J. Biochem., **25:** 153.

2. Massey, D. E. and Smyth, D. G. (1975): J. Biol. Chem. **250:** 6288.

3. Miyachi, Y. Vaitukais, J. L. Nieschlag, E. and Lipsett, M. B. (1972): J. Clin. Endocr.,**34:** 23.

4. Smyth, D. G., Markussen, J. and Sundby, F. (1974): Nature(Lond), **248:** 151.

5. Tager, H. S., Emdin, S.O., Clark, J. L. and Steiner, D. F. (1973): J. **248:** 3476.

6. Tager, H. S. and Steiner, D. F. (1972): J. Biol. Chem., **247,** 7936.

7. Yanaihara, N., Sakagami, M., Sakura, N., Iizuka, Y., Nishida, T., Hashimoto, T. and Yanaihara, C. (1977): In: Deabetes. P116 Ed J.S.Bajaj. Excerpta Media, Amsterdam.

8. Yanaihara, N., Nishida, T., Sakagami, M., and Yanaihara, C. (1977): In: Peptide Chemistry 1976, p85 Ed T. Nakajima. Protein Research Foundation, Osaka.

9. Yanaihara, N., Yanaihara, C., Sakagami, Sakura, N., Hashimoto, T. and Nishida, T (1978): , **27**, (Suppl 1) 149.

10. Yanaihara, C., Ozaki, J., Nishida, T , Iizuka, Y., Sato H., Yanaihara, N., and Kaneko, T.. (1979): Eds. S. Baba, T. Kaneko, N. Yanaihara, p87. Excerpta Medica, Amsterdam.–Oxford

11. Luo, W. Q., Kanno, T., Winarto, A, Iwanaga, T., Li., J., Futai, Y. Yanaihara, C., and Yanaihara, N.(1998) : Biomed, Res., **19,** 127

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