COSMO BIO CO., LTD.
Inspiration for Life Science

Mouse C-Peptide I EIA Kit

Cat. No. YII-YK011-EX

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

YII-YK011-EX　Mouse C – Peptide I EIA

Ⅰ. Introduction

This enzyme immunoassay (EIA)kit is a stable and convenient assay system for mouse C-peptide I 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 mouse C-Peptide I as standard antigen and biotinylated mouse C-Peptide I as labeled antigen. The kit contains specific polyclonal antibody recognized to the amino acid sequence of mouse C-Peptide.
Now, we have still tried to develop mouse C-peptide II EIA kit and mouse C-peptide I+II kit in our laboratory.

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of mouse 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.　Mouse C-Peptide I standard is highly purified synthetic product (purity: higher than 98%) and biotinylated peptide is stable because N –biotinylglycylglycyl Mouse C-Peptide I is used for it.

< Specificity >
The EIA kit shows each cross reactivity of 6.3% to mouse C-Peptide II, 6.5% to mouse insulin, 156.4% to rat C-Peptide I, 114.6% to rat C-Peptide II and 7.4% to rat insulin and shows no cross reactivity to human and dog C-Peptide.

< Test　Principle >
This EIA kit for determination of mouse C-Peptide I in plasma and urine samples is based on a competitive enzyme immunoassay using combination with highly specific antibody to mouse C-Peptide I　and biotin – avidin affinity system. The 96 wells plate is coated with goat anti rabbit IgG and C-Peptide I standard or samples, biotinylated mouse C-Peptide I and rabbit anti mouse C-Peptide I antibody are added to the wells for competitive immunoreaction.　After rinsing out excess mouse C-Peptide I, HRP labeled streptoavidins are added to bind to the antigen-antibody complex so that HRP labeled streptoavidin - biotinylated mouse 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 mouse C-Peptide I 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 mouse C-Peptide I(50ng)
3. Labeled antigen	lyophilized	1 vial	Biotinylated mouse C-Peptide I
4. C-Peptide antibody	liquid	1 bottle (6 mL)	Rabbit anti mouse C-Peptide I
5. SA-HRP solution	liquid	1 bottle (12 mL)	HRP labeled streptoavidin
6. Substrate buffer	liquid	1 bottle (26 mL)	0.015% Hydrogen Peroxide
7. OPD tablet	tablet	2 tablets	o-Phenylenediamine hydrochloride
8. Stopping solution	liquid	1 bottle (12 mL)	1M-H_2SO_4
9. Buffer solution	liquid	1 bottle (20 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 492nm

2) Rotator for microtitration plate

3) Washing device for microtitration plate and dispenser for approximately 0.35 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 mouse C-Peptide I 50ng/vial) with 1mL of buffer solution, which affords 50 ng/mL standard solution. 0.1ml of the reconstituted standard solution is diluted with 0.2 mL of buffer solution, that yields 16.667 ng/mL standard solution. 0.1mL of 16.667 ng/mL standard solution is diluted with 0.2 mL of the buffer solution, that makes 5.556 ng/mL standard solution. Repeat the dilution to make each standard of 1.852, 0.617 ng/mL. Buffer solution is used as 0 ng/mL.

2) Preparation of labeled antigen:
Reconstitute labeled antigen with 11mL of buffer solution.

3) Preparation of substrate solution:
Resolve OPD tablet with 12 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 to room temperature (20 - 30°C) before beginning the test at least for one hour.

2. Fill 25μL of each of standard solutions (0, 0.617, 1.852, 5.556, 16.667, 50 ng/mL) or samples into wells first, then add 100μL of labeled antigen and finally introduce 50μL of C-Peptide I antibody into the wells .

3. Cover the plate with adhesive foil and incubate it at 4°C for 16 - 18 hours.

4. Incubate it 1 hour at room temperature. During the incubation, the plate should be rotated with a plate rotator.

5. 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.

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

7. Cover the plate with adhesive foil and incubate it at room temperature for 1 hour. During the incubation, the plate should be rotated with a plate rotator.

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

9. Add 100μL of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.

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

11. Read the optical absorbance of the wells at 492nm.

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

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

Ⅴ. Notes

1. Plasma and serum 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 or serum samples. EDTA plasma is recommended to use for the determination.

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 4°C and color reaction, the test plate should be rotated gently by plate rotator to promote immunoreaction.

7. The plate can be used for separately twice, then reconstituted reagents(standard and labeled antigen) should be stored at 4°C within 1 week and stored at or less than -30°C more than 1 week.

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.

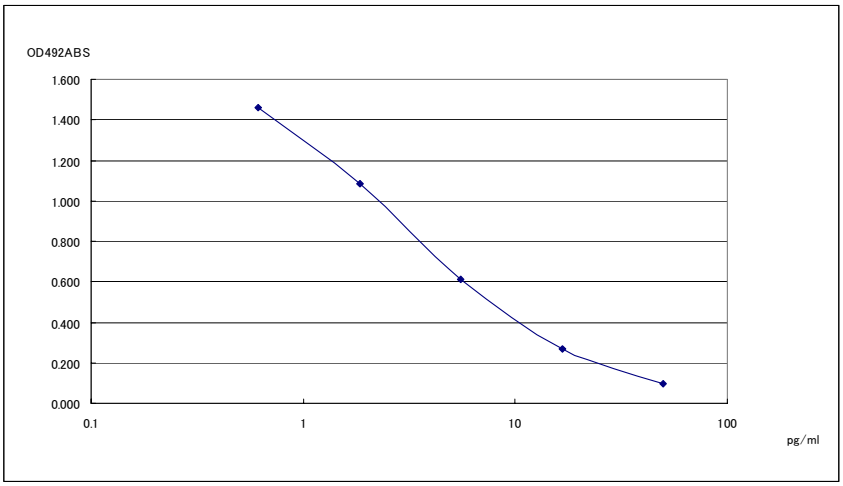
10. To quantitate accurately, always run a standard curve when testing samples.

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



< Precision and reproducibility >

- Intra-assay CV(%) : serum 3.1 ~ 4.9
  : plasma 5.6 ~ 7.5
  : urine 3.4 ~ 4.6
- Inter-assay CV(%) : serum 4.7 ~ 8.8
  : plasma 4.8 ~ 8.1
  : urine 5.3 ~ 11.3

< Assay range >

0.617 – 50 ng/mL

< Analytical recovery >

Sample No.	Mouse C-Peptide I added ng/mL	Observed ng/mL	Expected ng/mL	Recovery %
Serum 1	0.00	1.97		
	1.85	3.82	3.82	100.0
	5.56	8.60	7.53	114.2
	16.67	23.03	18.64	123.6
Serum 2	0.00	1.89		
	1.85	3.73	3.74	99.7
	5.56	7.94	7.44	106.7
	16.67	22.22	18.55	119.8

Sample No.	Mouse C-Peptide I added	Observed	Expected	Recovery
	ng/mL	ng/mL	ng/mL	%
Serum 3	0.00	2.32		
	1.85	4.06	4.17	97.3
	5.56	8.40	7.87	106.7
	16.67	22.38	18.98	117.9
Plasma 1	0.00	1.82		
	1.85	3.64	3.67	99.2
	5.56	7.55	7.37	102.4
	16.67	22.95	18.49	124.1
Plasma 2	0.00	1.97		
	1.85	3.42	3.60	95.0
	5.56	7.87	7.31	107.7
	16.67	22.30	18.42	121.1
Plasma 3	0.00	1.34		
	1.85	3.15	3.19	98.6
	5.56	7.17	6.89	104.0
	16.67	19.73	18.01	109.6
Plasma 4	0.00	1.71		
	1.85	3.60	3.57	100.9
	5.56	7.53	7.27	103.5
	16.67	18.45	18.38	100.4
Urine 1	0.00	1.66		
	1.85	2.47	2.51	98.4
	5.56	6.57	6.21	105.8
	16.67	19.17	17.32	110.7
Urine 2	0.00	1.25		
	1.85	2.91	3.10	93.7
	5.56	6.83	6.80	100.4
	16.67	20.09	17.92	112.1
Urine 3	0.00	2.24		
	1.85	3.59	4.08	88.0
	5.56	7.61	7.79	97.7
	16.67	22.34	18.90	118.2

Ⅶ. Stability and Storage

< Storage >	Store all of the components at 2 to 8°C.
< Shelf life >	12 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

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