YK012　Mouse C-Peptide II EIA

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Contents

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

YK012 Mouse C - Peptide II EIA

Ⅰ. Introduction

This enzyme immunoassay (EIA) kit is a stable and convenient assay system for mouse C-peptide II in its plasma, serum and urine. The processing of proinsulin, which occurs within the B cell, yields insulin and C-peptide. The 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 II as standard and biotinylated mouse C-Peptide II as labeled antigen. The kit contains specific polyclonal antibody recognized to the amino acid sequence of mouse C-Peptide II.

We have already developed Mouse C-Peptide I EIA kit (YK011) and Mouse C-Peptide (total C-Peptide) kit (YK013) in our laboratory. These C-Peptide series will support C-Peptide researches as a specifically useful tool.

YK012 Mouse C-Peptide II EIA Kit

- ▼ The assay kit can measure mouse C-Peptide II in the range of 0.412-100 ng/mL.
- ▼ The assay completes within 16-18 hr. + 2.5 hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate.
- ▼ Test sample: mouse plasma, serum or urine
  Sample volume: 25 µL
- ▼ The 96-well plate in kit is consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV (%) serum 4.12-5.66
  Inter-assay CV (%) serum 2.72-10.62
- ▼ Stability and Storage
  Store all of the components at 2-8℃.
  This kit is stable under the condition for 24 months from the date of manufacturing.
  The expiry date is described on the label of kit.

Contents

1) Antibody coated plate
2) C-Peptide II standard
3) Labeled antigen
4) C-Peptide II 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 EIA kit is used for quantitative determination of mouse C-Peptide II in its plasma, serum & urine samples. The kit is characterized for sensitive quantification, high specificity and no influences with other components in samples. Mouse C-Peptide II standard is a highly purified synthetic product (purity: higher than 98%).

< Specificity >

The EIA kit shows following cross reactivity of 0.091% to mouse C-Peptide I, 0.34% to rat C-Peptide I, 63.3% to rat C-Peptide II and 1.8% to rat insulin and no cross reactivity to human and dog C-Peptide.

< Test Principle >

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

III. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	Microtiter plate	1 plate (96 wells)	Goat anti rabbit IgG
2.	C-peptide II Standard	Lyophilized	1 vial (100 ng)	Synthetic mouse C-Peptide II
3.	Labeled antigen	Lyophilized	1 vial	Biotinylated mouse C-Peptide II
4.	C-Peptide II antibody	Liquid	1 bottle (6 mL)	Rabbit anti mouse C-Peptide II
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 dihydrochloride
8.	Stop solution	Liquid	1 bottle (12 mL)	1M H_2SO_4
9.	Buffer solution	Liquid	1 bottle (25 mL)	Phosphate buffer
10.	Washing solution (concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
11.	Adhesive foil		3 pieces	

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 II standard (lyophilized mouse C-Peptide II 100 ng/vial) with 1 mL of buffer solution, which affords 100 ng/mL standard solution. The 0.1 ml of the reconstituted standard solution is diluted with 0.2 mL of buffer solution that yields 33.33ng/mL standard solution. The 0.1 mL of 33.33 ng/mL standard solution is diluted with 0.2 mL of the buffer solution, that makes 11.11 ng/mL standard solution. Repeat the dilution to make each standard solution of 3.704, 1.235, and 0.412 ng/mL. Buffer solution is used as 0 ng/mL.

2. Preparation of labeled antigen solution:
   Reconstitute labeled antigen with 12 mL of buffer solution.

3. Preparation of substrate solution:
   Resolve one 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. Bring all the reagents to room temperature (20-30°C) before beginning the test.

2. Fill 25 µL of each of standard solutions (0, 0.412, 1.235, 3.704, 11.11, 33.33, 100 ng/mL) or samples into wells first, then add 100 µL of labeled antigen and finally introduce 50 µL of C-Peptide II antibody into the wells.

3. Cover the plate with adhesive foil and incubate it at 4°C for 16 -18 hours. (Still, shaker not need)

4. After 4°C incubation, incubate it for 1 hour at room temperature. During the incubation, the plate should be shaken with a microtiter plate shaker.

5. Take off the adhesive foil, aspirate the solution in the wells and wash the wells 3 times with approximately 0.35 mL/well of washing solution.

6. Pipette 100 µL 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 shaken with plate shaker.

8. Take off the adhesive foil, aspirate and wash the wells 5 times with approximately 0.35 mL/well of washing solution.

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

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

11. 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 II concentrations in samples from the corresponding absorbance values.

Ⅴ. Notes

1. Plasma, serum or urine 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 -30°C. Avoid repeated freezing and thawing of samples. EDTA-2Na additive blood collection tube is recommended for the plasma.

2. C-Peptide II standard, labeled antigen, substrate solution should be prepared immediately before use. The kit can be used for separately. In that case, reconstituted reagents (standard and labeled antigen) should be stored at or below than -30°C if be used within two weeks.

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

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

5. When sample value exceeds 100 ng/mL, it needs to be diluted with buffer solution to a proper concentration.

6. During incubation except 4°C and color reaction, the test plate should be shaken gently by microtiter plate shaker to promote immunoreaction.

7. Perform all the determination in duplicate.

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

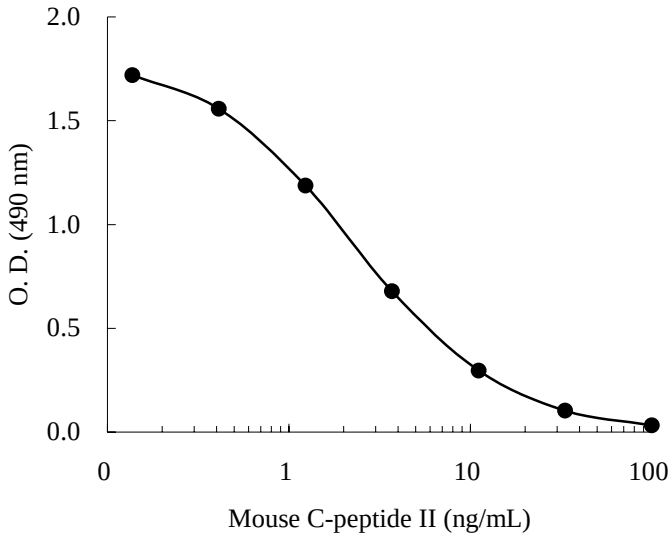
9. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

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

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

Ⅵ. Performance Characteristics

A typical standard curve



< Precision and reproducibility >

- Intra-assay CV (%) : Serum 4.12 ~ 5.66
- Inter-assay CV (%) : Serum 2.72 ~ 10.62

< Assay range >

0.412 ～100 ng/mL

< Analytical recovery >

Serum

Added (ng/mL)	Recovery (%)	SD（n=5）	CV (%)
0.520	107.624	4.846	4.503
1.042	110.917	4.044	3.646
2.083	108.311	3.990	3.684
4.167	120.291	4.683	3.893
8.333	116.770	8.377	7.174
16.667	114.817	5.646	4.918

Plasma

Added (ng/mL)	Recovery (%)	SD（n=5）	CV (%)
0.520	100.970	8.471	8.390
1.042	101.383	8.006	7.897
2.083	109.568	3.734	3.408
4.167	110.418	4.569	4.137
8.333	118.733	5.719	4.816
16.667	122.243	3.077	2.517

Urine

Added (ng/mL)	Recovery (%)	SD（n=4）	CV (%)
0.412	100.325	4.591	4.576
1.235	98.317	2.945	2.995
3.704	100.773	6.110	6.063
11.110	101.687	7.102	6.984
33.330	101.184	12.910	12.759

Remark: The mouse urine was collected within 24 hours and diluted 10 folds with assay buffer before the test.

Ⅶ. Stability and Storage

< Storage >	Store all of the components at 2 to 8°C.
< Shelf life >	This kit is stable under the condition for 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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