YK013　Mouse C-peptide　EIA

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

YK013 Mouse C-peptide EIA

Ⅰ. Introduction

This enzyme immunoassay (EIA) kit is a stable and convenient assay system for mouse C-peptide (mouse C-peptide I+II) in its serum and plasma.

The processing of proinsulin, which occurs within the pancreatic 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.

This EIA kit includes synthetic mouse C-peptide II as standard antigen and biotinylated mouse C-peptide II as labeled antigen. The kit also contains specific polyclonal antibody recognizing mouse C-peptide I and mouse C-peptide II equivalently.

We have already developed EIA kits which can measure specifically mouse C-peptide I (YK011) and C-peptide II (YK012), respectively and now mouse C-peptide (I+II) kit (YK013) is developed for measuring C-peptide I and C-peptide II together. Namely this kit is useful for measuring total C-peptide level in mouse blood.

YK013 Mouse C-peptide EIA Kit

- ▼ This assay kit can measure mouse C-peptide (I+II) within the range of 0.412-100 ng/mL
- ▼ The assay is completed within 18-20 hr + 1.5 hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate
- ▼ Test sample: mouse plasma and serum
  Sample volume: 25 μL
- ▼ The 96-well plate of this kit consists of 12 8-wells strips, so that divided use by the strips is possible at user's option.
- ▼ Precision and reproducibility
  Intra-assay CV (%) : 1.4-3.1
  Inter-assay CV (%) : 4.2-8.1
- ▼ Stability and Storage
  Store all the components at 2-8℃.
  The kit is stable under the condition for 24 months from the date of manufacturing.
  The expiry date is stated on the package.

Contents

1) Antibody Coated Plate
2) Standard
3) Labeled Antigen
4) Specific Antibody
5) SA-HRP Solution
6) TMB Substrate
7) Reaction Stopping Solution
8) Buffer solution
9) Concentrated Wash Solution
10) Adhesive Foil

Ⅱ. Characteristics

This EIA kit is used for quantitative determination of total mouse C-peptide, i.e. C-peptide I+II, in its serum and plasma samples. The kit is characterized by its sensitive quantification and high specificity. In addition, it is not influenced by other constituents in samples. Standard antigen, mouse C-peptide II of this kit is a highly purified synthetic product (purity: higher than 98%).

< Specificity >

This EIA kit shows 93.9% crossreactivity to mouse C-peptide I, 100% to mouse C-peptide II, 22.9% to rat C-peptide and 48.2% to rat C-peptide II. It shows below 2% crossreactivity to mouse insulin and no crossreactivity to human, dog and pig C-peptide.

< Assay principle >

This EIA kit for determination of mouse C-peptide in serum and plasma is based on a competitive enzyme immunoassay using combination of highly specific antibody to mouse C-peptide (I+II) and biotin-avidin affinity system. To the wells of the plate coated with goat anti rabbit IgG, labeled antigen, standard antigen or samples and rabbit anti mouse C-peptide antibody are added for competitive immunoreaction. After incubation and plate washing, horse radish peroxidase (HRP) labeled streptavidin (SA) is added to form HRP labeled SA-biotinylated antigen-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by 3,3'5,5'-tetramethyl benzidine (TMB) and the concentration of mouse C-peptide is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody Coated Plate	Microtiter plate	1 plate (96 wells)	Goat anti rabbit IgG antibody
2.	Standard	Lyophilized	1 vial (50 ng)	Synthetic mouse C-peptide II
3.	Labeled Antigen	Lyophilized	1 vial	Biotinylated mouse C-peptide II
4.	Specific Antibody	Liquid	1 bottle (6 mL)	Rabbit anti mouse C-peptide antibody
5.	SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP labeled SA
6.	TMB Substrate	Liquid	1 bottle (12 mL)	3,3',5,5'-tetramethyl benzidine (TMB)
7.	Reaction Stopping Solution	Liquid	1 bottle (12 mL)	1M H_2SO_4
8.	Buffer Solution	Liquid	1 bottle (25 mL)	Tris-HCl/saline buffer
9.	Concentrated Wash Solution	Liquid	1 bottle (25 mL)	Concentrated saline
10.	Adhesive Foil		3 pieces	

Ⅳ. Method

< Equipment required >

1. Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450nm.

2. Washing device for microtiter plate and dispenser with aspiration system.

3. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips.

4. Polypropylene or glass test tubes for preparation of standard solution.

5. Graduated cylinder (500 mL)

6. Distilled or deionized water.

< Preparatory work >

1. Preparation of standard solution:
   Reconstitute the Standard (lyophilized mouse C-peptide II 50 ng/vial) with 0.5 mL of Buffer Solution, which affords 100 ng/mL standard solution. The reconstituted standard solution (0.1 mL) is diluted with 0.2 mL of Buffer Solution, which yields 33.33 ng/mL standard solution. Repeat the dilution procedure to make each of 11.11, 3.704, 1.235 and 0.412 ng/mL standard solutions. Buffer Solution itself is used as 0 ng/mL.

2. Preparation of labeled antigen solution:
   Reconstitute Labeled Antigen with 8 mL of Buffer Solution.

3. Preparation of washing solution:
   Dilute 25 mL of Concentrated Wash Solution to 500 mL with distilled or deionized water.

4. Other reagents are ready for use.

< Procedure >

1. Bring all the reagents and samples to room temperature (20-30°C) at least 1 hour before starting assay.

2. Add 0.35mL/well of washing solution into the wells of the plate and keep it for about 30 seconds, and then aspirate the solution. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

3. Fill 50µL of labeled antigen solution first, then add 25µL each of standard solutions (0, 0.412, 1.235, 3.704, 11.11, 33.33 and 100 ng/mL) or samples into wells and finally introduce 50µL of specific antibody.

4. Cover the plate with Adhesive Foil and incubate it at room temperature for 18-20 hours.

5. After incubation, 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. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

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

7. Cover the plate with Adhesive Foil and incubate it at room temperature for 1 hour.

8. Take off the Adhesive Foil, aspirate the solution in the wells and then wash the wells 5 times with approximately 0.35 mL/well each of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

9. Add 100µL of TMB Substrate into each of the wells, cover the plate with Adhesive Foil and keep it for 30 minutes at room temperature in a dark place for color reaction.

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

11. Read optical absorbance of the solution in the wells at 450 nm. Calculate mean absorbance values of standard solutions and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard antigen; ordinate: absorbance value). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

Ⅴ. Notes

1. EDTA-2Na additive blood collection tube is recommended for plasma sample collection. It is recommended that serum and plasma samples should 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.

2. Standard solution and labeled antigen solution should be prepared immediately before use.

3. Diluted washing solution is stable for 6 months at 2-8°C.

4. Pipetting operations may affect precision of the assay. Pipette standard solution or samples into each well of the plate precisely. Use clean test tubes and vessels in assay, and new tip must be used for each sample and standard solution or standard diluting preparation to avoid cross contamination.

5. Perform all the determination in duplicate.

6. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagents (standard and labeled antigen solutions) should be stored at or below -30°C.

7. For accurate quantification, plot a standard curve for each assay.

8. Color reaction by TMB substrate must be carried in a dark place.

9. Read optical absorbance of reaction solution in the wells immediately after stopping color reaction.

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

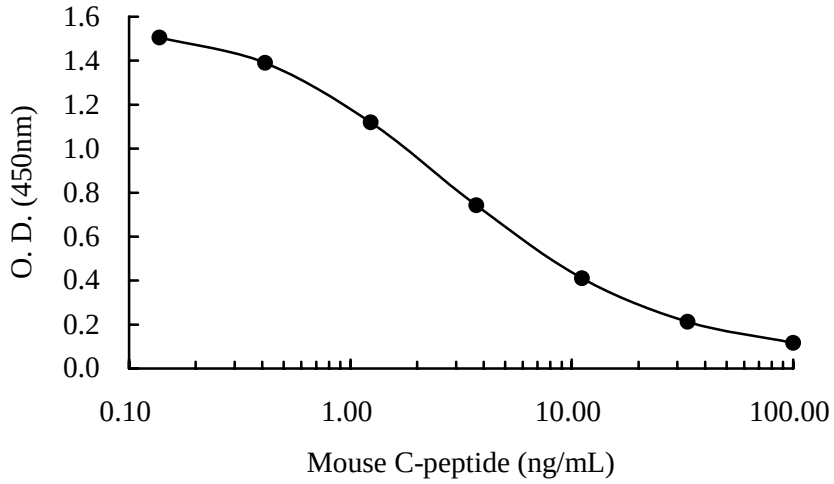
11. Satisfactory performance of assay is guaranteed only when reagents in combination pack with identical lot number are used.

Ⅵ. Performance Characteristics

<Assay range> 0.412-100 ng/mL

If a sample concentration below 0.412 ng/mL is predicted, standard curve may be further set up a lower detection limit by using 0.137 ng/mL standard solution which can be prepared by 3-fold dilution of 0.412 ng/mL standard solution. In such case, however, assay precision may not be so excellent as that of the cases between 0.412 and 100 ng/mL.

A typical standard curve



< Precision and reproducibility >

Intra-assay CV (%) : 1.4-3.1

Inter-assay CV (%) : 4.2-8.1

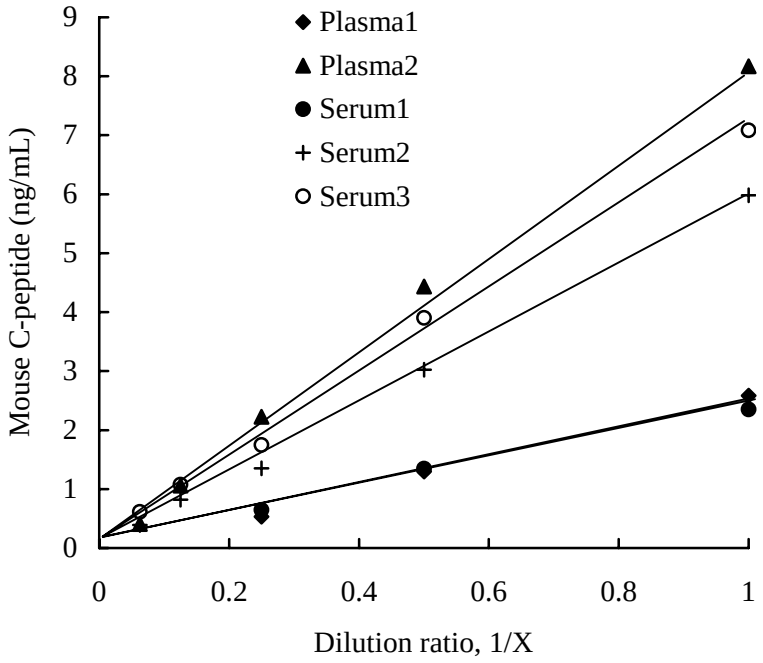
(The precision and reproducibility tests were tested using different concentrations of mouse serum samples.)

< Analytical recovery >

Mouse plasma (n=4) 105.7-112.6%

Mouse serum (n=4) 98.4-107.4%

<Dilution test>



Ⅶ. Stability and Storage

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

< Shelf life > The kit is stable under the condition for 24 months from the date of manufacturing. The expiry date is stated on the package.

< Package > For 96 tests per one kit.

Ⅷ. References

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