

YK011 Mouse C-Peptide I EIA

FOR LABORATORY USE ONLY

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

YK011 Mouse C-Peptide I EIA

. 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. 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 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 I.

We have already developed Mouse C-Peptide II EIA kit (YK012) and Mouse C-Peptide (total C-peptide) kit (YK013) in our laboratory. This C-Peptide series will support C-Peptide researches as specifically useful tools.

YK011 Mouse C-Peptide I EIA Kit	Contents
The assay kit can measure mouse C-Peptide I in the range of 0.617-50 ng/mL	1) Antibody coated plate
The assay completed within 16-18 hr.+ 2.5 hr.	2) C-Peptide I standard
With one assay kit, 42 samples can be measured in duplicate	3) Labeled antigen
Test sample: mouse plasma, serum or urine	4) C-Peptide I antibody
Sample volume: 25 μ L	5) Buffer solution
The 96-well plate in kit is consisted by 8-wells strips. The kit can be used separately.	6) SA-HRP solution
Precision and reproducibility	7) Substrate buffer
Intra-assay CV (%)	8) OPD tablet
Serum 3.1-4.9, plasma 5.6-7.5, urine 3.4-4.6	9) Stopping solution
Inter-assay CV (%)	10) Washing solution (concentrated)
Serum 4.7-8.8, plasma 4.8-8.1, urine 5.3-11.3	11) Adhesive foil
Stability and Storage	
Store all of the components at 2~8°C	
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.	

. Characteristics

This kit is used for quantitative determination of mouse C-Peptide I 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 I standard of this kit is a highly purified synthetic product (purity: higher than 98%).

< Specificity >

The EIA kit shows 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. The kit also can be used for measure rat C-Peptide, but the standard is different, we strong recommend using our product YK010 for measuring rat C-Peptide.

< Test Principle >

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

. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	MTP ^{*1}	1 plate (96 wells)	Goat anti rabbit IgG
2. C-Peptide I standard	lyophilized	1 vial (50 ng)	Synthetic mouse C-Peptide I
3. Labeled antigen	lyophilized	1 vial	Biotinylated mouse C-Peptide I
4. C-Peptide I antibody	liquid	1 bottle (6 mL)	Rabbit anti mouse C-Peptide I
5. Buffer solution	liquid	1 bottle (20 mL)	Phosphate buffer
6. SA-HRP solution	liquid	1 bottle (12 mL)	HRP labeled streptoavidin
7. Substrate buffer	liquid	1 bottle (26 mL)	0.015% Hydrogen Peroxide
8. OPD tablet	tablet	2 tablets	o-Phenylenediamine dihydrochloride
9. Stopping solution	liquid	1 bottle (12 mL)	1M H ₂ SO ₄
10. Washing solution (Concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
11. Adhesive foil		3 pieces	

MTP^{*1}..... Microtiter plate

. 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 I standard (lyophilized mouse C-Peptide I 50 ng/vial) with 1 mL of buffer solution, which affords 50 ng/mL standard solution. The 0.1 ml of the reconstituted standard solution is diluted with 0.2 mL of buffer solution that yields 16.667 ng/mL standard solution. The 0.1 mL 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 solution of 1.852, 0.617 ng/mL. Buffer solution is used as 0 ng/mL.
2. Preparation of labeled antigen solution:
Reconstitute labeled antigen with 11 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 and samples to room temperature (20~30°C) before beginning the test at least for 1 hour.
2. Fill 25 μL of each of standard solutions (0, 0.617, 1.852, 5.556, 16.667, 50 ng/mL) or samples into wells first, then add 100 μL of labeled antigen solution and finally introduce 50 μL 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. (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. 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 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 a plate shaker.
8. Take off the adhesive foil, aspirate and wash the wells 5 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.
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 color reaction.
11. Read the optical absorbance of the solution in the wells at 490 nm. Calculate mean absorbance values of standard solution and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

. Notes

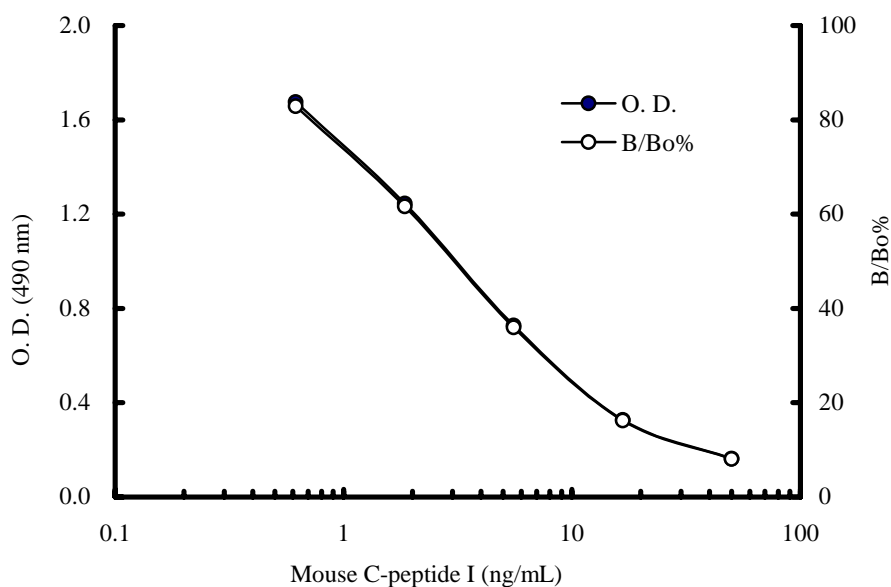
1. Plasma, serum or urine samples must be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30°C . Avoid repeated freezing and thawing of samples. EDTA-2Na additive blood collection tube is recommended for the plasma collection.
2. C-Peptide I standard, labeled antigen, substrate solution should be prepared immediately before use. The kit can be used for separately twice. In that case, reconstituted reagents (standard and labeled antigen) should be stored at 4°C if it would be used within one week. If reconstituted reagents be used later more than one week, it should be stored at or below -30°C .
3. During storage of washing solution (concentrated) at $2\sim 8^{\circ}\text{C}$, precipitates may be observed, however they will be dissolved when diluted.
4. Pipetting operations may affect the precision of the assay, pipette standard solutions or samples into each well of plate precisely. Using clean test tubes or vessels in assay, and new tip must be used for each sample and standard to avoid cross contamination.
5. When concentration of C-Peptide 1 in sample is expected to exceed 50 ng/mL , the sample needs to be diluted with buffer solution to a proper concentration. It should be diluted $10\sim 100$ fold by buffer solution if the sample is urine.
6. During incubation except the case at 4°C incubation and color reaction, the plate should be shaken gently with a microtiter plate shaker to promote immunoreaction.
7. Perform all the determination in duplicate.
8. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.
9. For accurate quantification, plot a standard curve for each assay.
10. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
11. Satisfactory performance of the test is guaranteed only when reagents in combination pack with identical lot number are used.

. Performance Characteristics

<Assay range> 0.617-50 ng/mL

If a sample concentration below 0.617 ng/mL is predicted, standard curve may be further set up a lower detection limit by using 0.206 ng/mL standard solution which can be prepared by 3-fold dilution of 0.617 ng/mL standard solution. In such case, however, assay precision may not be so excellent as that of the cases between 0.617 and 50 ng/mL.

Typical standard curve



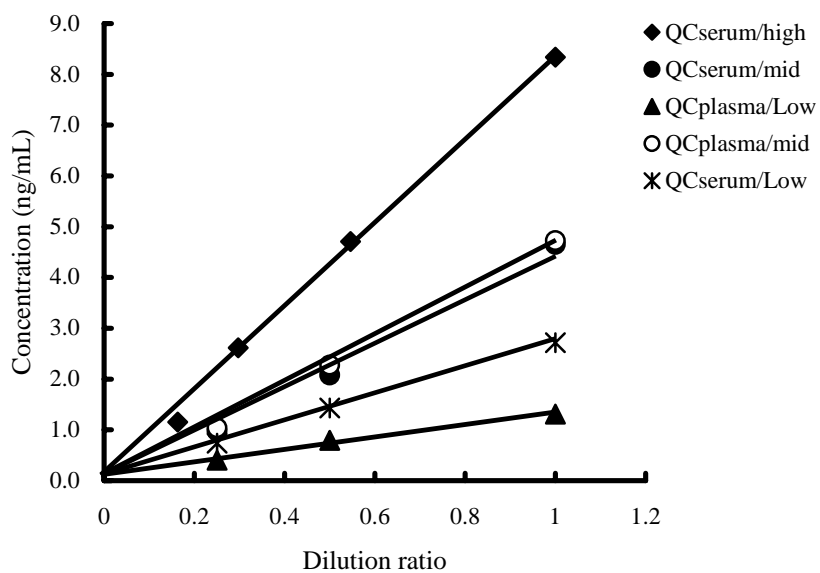
< Precision and reproducibility >

- 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

<Dilution of mouse serum/plasma>

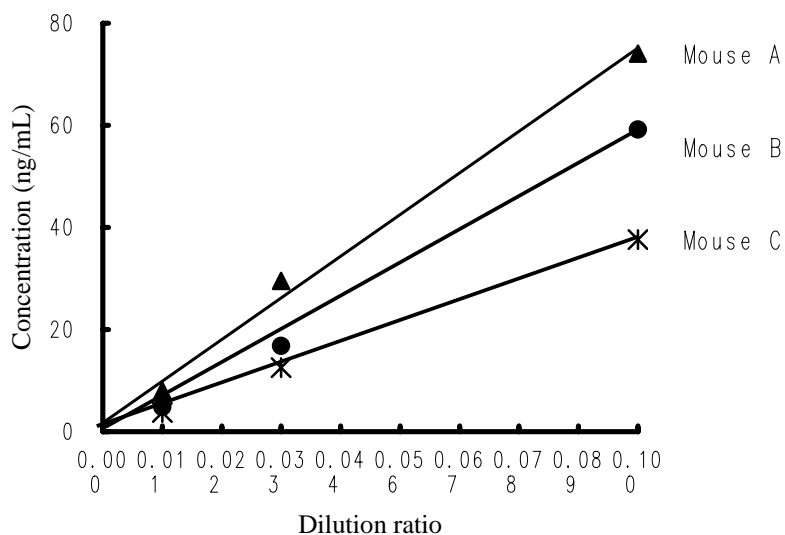
Dilution curves of mouse serum/plasma



serum/plasma (Low: no C-Peptide I added, Mid & High: C-Peptide I added)

<Dilution of mouse urine>

Dilution curves of mouse 24hours urine



Remark: The mouse urine was collected within 24 hours and diluted 10, 30, 90 folds with assay buffer before the test.

< Analytical recovery >

Sample No.	C-Peptide added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Mouse serum No.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
Mouse serum No.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
Mouse serum No.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
Mouse EDTA plasma No. 4	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
Mouse EDTA plasma No. 5	0.00	1.75		
	1.85	3.42	3.60	95.0
	5.56	7.87	7.31	107.7
	16.67	22.30	18.42	121.1
Mouse EDTA plasma No. 6	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
Mouse EDTA plasma No. 7	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
Mouse Urine No.8	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
Mouse Urine No.9	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
Mouse Urine No.10	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

. Stability and Storage

- < Storage > Store all of 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 described on the label of kit.
- < Package > For 96 tests per one kit including standards

. References

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