Rat C-peptide ELISA Kit (U type)

Research Reagent

Please, read this instruction carefully before use.

This is an ELISA (Enzyme Linked Immunosorbent Assay) kit for measurement of rat C-peptide with high sensitivity using Sandwich assay principle.

[Advantage]

(1) Rat C-peptide ELISA Kit can measure C-peptide rapidly (5 hours).
(2) Rat C-peptide ELISA Kit can measure with a small sample volume (10μl).
(3) Rat C-peptide ELISA Kit uses an ecologically excellent preservative.
(4) Every reagent is provided in liquid form and ready to use.
(5) Excellent precision and reproducibility.

[Components]

Reagents	Amounts
(A) Anti-C-peptide-coated plate	96 wells (8x12) / 1 plate
(B) Standard C-peptide solution (6000pg/ml)	500μl / 1 vial
(C) Buffer solution	60ml/1 vial
(D) Biotin-conjugated anti-C-peptide	100μl/ 1 vial
(E) HRP-conjugated streptavidin	100μl/ 1 vial
(F) Chromogenic substrate reagent(TMB)	12ml/ 1 vial
(H) Reaction stopper (1M H_2SO_4)	12ml/ 1 vial
(I) Concentrated washing buffer(10x)	100ml/ 1 bottle

[Assay samples]

Rat serum or plasma. 10μl/well

[Purpose of the assay]

Measurement of C-peptide in rat serum or plasma.

[Assay range]

30~3000 pg/ml

According to the standard procedure where the samples are diluted 5 times, practical assay range is 150~15,000pg/ml

[Assay operation]

1. Equipments necessary but not included in the kit.
   (1) Micropipette (we recommend to use a micropipette prepared for delivering 0.5 ~ 10μl .)
   (2) Microplate washing apparatus (a microplate washer or a nozzled flashing bottle)
   (3) A microplate reader (A densitometer for microplate).
2. Preparation of reagents

(4) Washing buffer: Dilute the concentrated washing buffer (I) to 10X with purified water.
(5) Biotin-conjugated anti-C-peptide (D) : Dilute to 1:100 with the buffer solution(C).
(6) HRP-conjugated streptavidin (E): Dilute to 1:100 with the buffer solution(C).
(7) Other reagents are used as they are.
(8) All the reagent solutions should be used after getting back to room temperature (20-25C).

3. An example of preparing standard solutions

Dilute the original standard solution (B) with the buffer solution to prepare 3,000pg/ml, then prepare lower standard solutions by serial dilution.

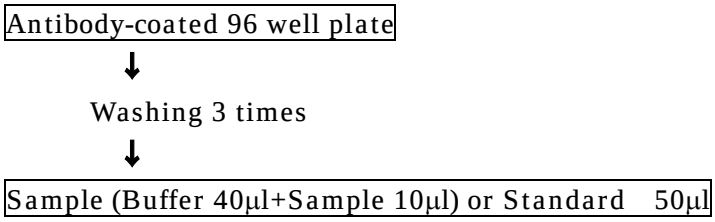
Concentration(pg/ml)	3,000	1,500	600	300	150	60	30	0
Std. Sol. (μl)	150(orig)	150*	150*	150*	150*	150*	150*	0
Buffer(μl)	150	150	225	150	150	225	150	150

*One rank higher standard solution

4. Assay procedure

1) Rinse the anti-C-peptide coated wells (A) by filling the washing buffer and discard 3 times, then strike the plate upside-down several times onto stacked sheets of paper towel, and remove the excess buffer.
2) Pipette 40μl of buffer solution into the wells for samples, then add 10μl of sample. Alternatively, if samples are already diluted to 5X, pipette 50μl of the diluted sample to each well, skipping the addition of buffer solution.
3) Pipette 50μl of the standard solution to the wells for preparing a standard curve.
4) Shake the plate gently on a plate shaker.
5) Incubate for 2 hours at room temperature (20-25C).
6) Discard the reaction mixture, then wash wells as described in (1).
7) Pipette 50μl of biotin-conjugated anti-C-peptide solution to all wells. Then shake gently on a plate shaker.
8) Incubate the plate for 2 hours at room temperature.
9) Discard the reaction mixture, then wash the plate as (1).
10) Pipette 50μl of HRP-conjugated streptavidin solution to all wells, and shake as (7).
11) Incubate for 30 minute at room temperature.
12) Discard the reaction mixture, and wash the plate as (1).
13) Pipette 50μl of the chromogenic substrate solution to wells, and shake as (7).
14) Let the plate stand for 30 minutes at room temperature.
15) Add 50 μl of the reaction stopper (H) to all wells and shake.
16) Measure the absorbance of each well at 450 nm (sub-wave length, 620nm) by a plate reader within 30 minutes.

[Summary of Assay Procedure]



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Shaking, and reaction for 2 hr. at room temp.

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Washing 3 times

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Biotin-conjugated anti-C-peptide 50μl

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Shaking, and reaction for 2 hr. at room temp

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Washing 3 times

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HRP-avidin 50μl

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Shaking, and reaction for 30min. at room temp

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Washing 3 times

↓

Chromogenic substrate solution 50μl

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Shaking, and reaction for 30min. at room temp.

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Reaction stopper (1M H_2SO_4) 50μl

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Shaking, and measurement of absorption at 450nm
(Sub-wave length 620nm)

Room temp.: 20~25C

[Calculation of C-peptide concentration]

(1) Prepare a standard curve using hemi-logarithmic section paper by plotting absorbance (Y-axis) against logarithm of C-peptide concentration (pg/ml) on X-axis. In case of manual reading of the standard curve, we recommend the use of logarithmic (both-way) section paper, and plotting logarithm of absorbance on Y axis and logarithm of standard concentration on X axis.

(2) Using the standard curve, read the C-peptide concentration of a sample from its absorbance. Multiply this value by 5 because the sample is diluted 5X in the standard procedure.

○ Though the assay range is very wide, in case the absorbencies of some samples are higher than that of the highest standard, please repeat the assay after proper dilution of samples with the buffer solution.

○ We recommend using 3rd order regression curve or 4 parameter method in computer calculation.

[Important notice in the treatments]

1. Treatment of assay samples

（1） Use serum or plasma samples obtained by ordinary standard method.
Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.

（2） Turbid samples or those containing insoluble materials should be centrifuged before assay and remove those materials.

（3） Measure the samples as soon as possible after sampling.

2. Storage of assay samples.

If assay samples have to be stored for a long period, freeze samples and store below –35C. Avoid repeated freezing and thawing.

3. Influence of interfering substances

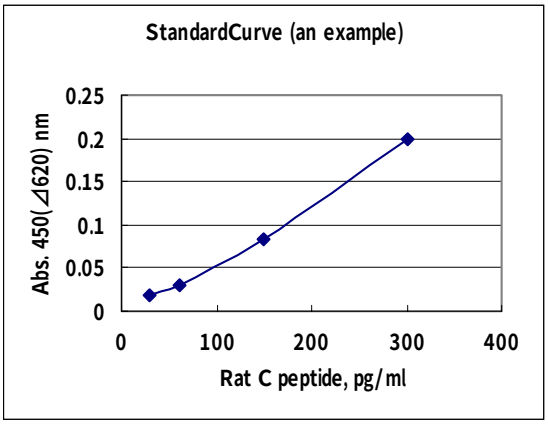
If presence of interfering substances is suspected, examine by a dilution test using more than 2 points.

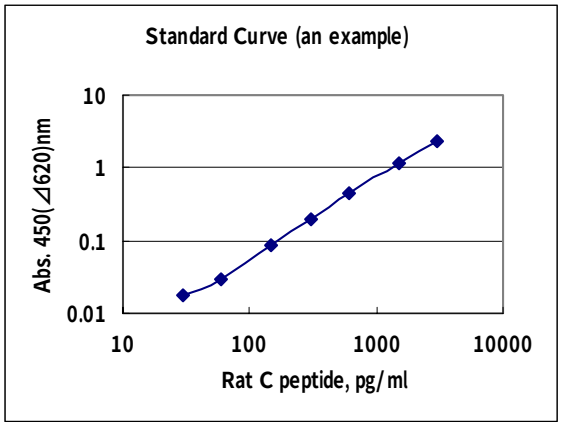
[Assay range and assay validation]

1. Assay range

As seen in a standard model shown, absorbance between 156~10,000pg/ml is 0.05 to 3.0.

[Model standard curves]





2. Specificity

In this assay kit we use the monoclonal antibodies which react both Rat C-peptide I and II.

Cross-reactivity

Species	Substance	Cross reactivity (%) *
Rat	C-peptide	100
	Insulin	Less than sensitivity
	Pro-Insulin	Less than sensitivity
Mouse	C-peptide	75
	Insulin	Less than sensitivity
Human	C-peptide	85
	Insulin	Less than sensitivity

	Pro-Insulin	Less than sensitivity
Pig	Insulin	Less than sensitivity
Cow	Insulin	Less than sensitivity

*Cross-reactivity was estimated with 15,000 pg/ml of the substance

3. Precision and reproducibility

（1） Within assay variation (2 samples, n=8)

Well/Sample	A	B
1	1015	214
2	1027	222
3	1038	211
4	1043	219
5	1029	232
6	1034	209
7	1039	231
8	1041	224
mean	1033	220
SD	9.60	8.51
CV (%)	0.93	3.86

Unit：pg/ml

（2） Reproducibility (3 samples, n = 4, 4 days)

Sample/day	Day 1	Day 2	Day 3	Day 4	mean	SD	CV (%)
C	1499	1435	1491	1458	1471	29.68	2.02
D	599	605	569	559	583	22.55	3.87
E	63.9	58.2	59.9	64.2	61.6	2.98	4.83

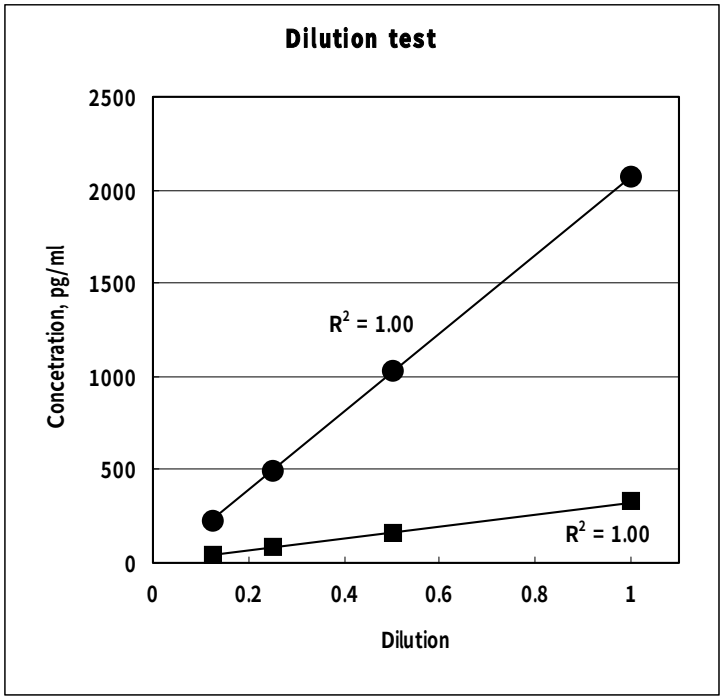
Unit：pg/ml

（3） Recovery test

Added	Found	Recovered	Recovery %
0.00	180	-	-
110	291	111	101
161	332	152	94.5
209	379	199	95.1
Added	Found	Recovered	Recovery %
0.00	360	-	-
263	616	256	97.1
526	909	549	104
658	1040	680	103

Unit：pg/ml

（4）Dilution test



（5）Blood levels in rats

Samples: Female rats Drl:CD (SD) 6 weeks of age, from Charles River Japan

Free access to food and water

Assay: n = 2

Animal No.	CP level, ng/ml
1	0.94
2	1.05
3	1.22
4	1.32
5	1.47
6	1.53
7	1.79
8	2.03
9	2.04
10	2.16
mean	1.55

[Statements and precaution]

1 The reagents included in this assay kit should be used only for research works.

2 The reagent solutions of the kit should be used principally immediately after reconstitution. Otherwise, keep them in a dark place with the temperature 2-8C, and use them within 3 days.

3 The reagents were prepared to give accurate results by their combination within the kit. So, do not combine the reagents in the kit of other lot number. Even the lot number is

the same, do not mix the reagents with those that have been preserved for some period.

4 Pipetting and dilution of the reagent solutions should be made accurately because these steps influence the assay precision.

5 Do no dry the assay plate to avoid denaturation of the coated antibody.

6 Measurement of the reaction time should be started from the pipetting of reagent to the first well.

7 Prepare the standard curve in each assay.

8 Dilution of the assay sample must be carried out using the buffer solution attached to the kit.

9 Storage condition for the kit should be strictly followed.

10 Be careful not to allow the reagent solutions of the kit to touch the skin and mucus. Especially be careful for the stopping solution because it is 1M sulfuric acid.

11 HRP-conjugated reagent solution, chromogenic substrate solution, and reaction stopper must be avoided from contacting with any metal.

12 In treating assay samples of animal origin, be careful for possible biohazards.

13 As the antibody-coated plate is module type of 8wells x 12 rows, each row can be separated by a cutter and used independently.

[Storage condition]

Store the kit at 2~8C (Do not freeze.)

[Term of validity]

6 month from production (Expiration date is indicated on the container)

[Unit of package]

96-wells/1 plate (Product code: AKRCP-030)

Shibayagi Co., Ltd.

1062-1 Ishihara Shibukawa, Gunma, Japan 377-0007

TEL.+81-279(25)0279, FAX.+81-279(23)0313

URL:http://www.shibayagi.co.jp/

E-mail:syc-info@shibayagi.co.jp