SHIBAYAGI	Mouse 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 mouse C-peptide with high sensitivity using Sandwich assay principle.

[Advantage]

(1) Rapid assay (total reaction time: 5 hours.).
(2) A small sample volume (10μl in standard assay procedure).
(3) An ecologically excellent preservative is used.
(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 mouse C-peptide solution (6000pg/ml)	500μl / 1 vial
(C)	Buffer solution	60ml/**1** vial
(D)	Biotin-conjugated anti-C-peptide	100μl/ 1 vial
(E)	Peroxidase-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 sample]

Mouse serum or plasma 10 μl/well (Standard procedure)

[Assay purpose]

Measurement of C-peptide in mouse serum or plasma samples, and in culture media.

[Assay range]

Assay range of the standard curve is 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 (a micropipette able to deliver sample volume with high precision.), and a pipette for repetitive dispensing.
   (2) Microplate washing apparatus (a microplate washer or a flashing bottle with nozzle).
   (3) A microplate reader (A densitometer for microplate).
2. Preparation of reagents
   (1) Washing buffer: Dilute the concentrated washing buffer (I) to 10X with purified water.
   (2) Biotin-conjugated anti-C-peptide (D) : Dilute to 100X with the buffer solution(C).
   (3) HRP-conjugated streptavidin (E): Dilute to 100X with the buffer solution(C).
   (4) Other reagents are used as they are.
   (5) 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 3000ng/ml, then prepare lower standard solutions by a dilution program shown below.

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

**Original standard soloution, *One rank higher standard solution

4. Assay procedure

1)	Remove the cover sheet of the microplate after getting back to room temperature.
2)	Rinse the anti-body coated wells (A) by filling the washing buffer and discard 3 times, then strike the plate upside-down onto folded several sheets of paper towel, and remove the excess buffer.
3)	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.
4)	Pipette 50μl of the standard solution to the wells for preparing a standard curve.
5)	Shake the plate gently on a plate shaker.
6)	Incubate for 2 hour at room temperature (20-25C).
7)	Discard the reaction mixture, and then wash wells as described in (2).
8)	Pipette 50μl of biotin-conjugated anti-C-peptide solution to all wells. Then shake gently on a plate shaker.
9)	Incubate the plate for 2 hour at room temperature.
10)	Discard the reaction mixture, and then wash the plate as (2).
11)	Pipette 50μl of HRP-conjugated avidin solution to all wells, and shake as (5).
12)	Incubate for 30 minute at room temperature.
13)	Discard the reaction mixture, and wash the plate as (2).

14)	Pipette 50μl of chromogenic substrate solution to wells, and shake as (5).
15)	Let the plate stand for 20 minutes at room temperature.
16)	Add 50 μl of the reaction stopper (H) to all wells and shake.
17)	Measure the absorbance of each well at 450 nm (sub-wave length, 620nm) by a plate reader within 30 minutes.

[Summary of Assay Procedure]

Antibody-coated 96 well plate

↓

Washing 3 times

↓

Sample (diluted) or Standard 50μl

↓

Shaking and reaction for 2 hr at room temp.

↓

Washing 3 times

↓

Biotin-conjugated anti-C-peptide 50μl

↓

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

↓

Washing 3 times

↓

HRP-conjugated avidin 50μl

↓

Shaking and reaction for 30 min at room temp.

↓

Washing 3 times

↓

Chromogenic substrate solution 50μl

↓

Shaking, and reaction for 30 min. at room temp

↓

Reaction stopper 1M H_2SO_4 50μl

↓

Shaking and measurement of absorbance
at 450nm(sub. 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.

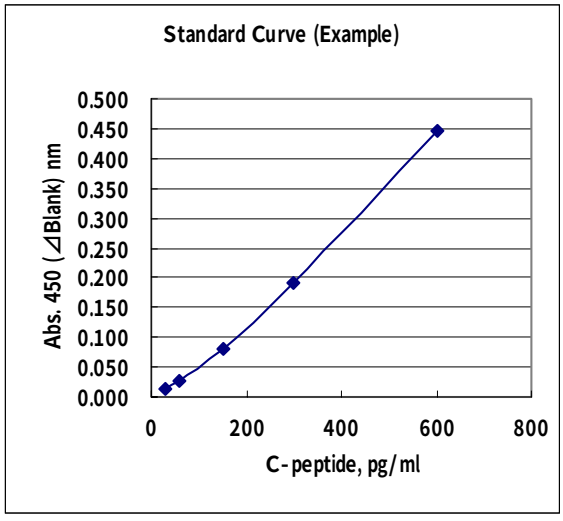
○ We recommend using 3rd order regression curve or 4 parameter method in computer calculation.

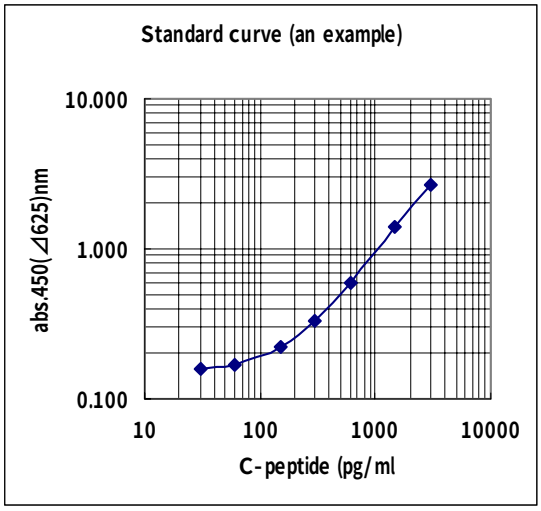
[Important notice in the treatments]

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. Model standard curves





2. Specificity

In this assay kits we used monoclonal antibodies that react both mouse C-peptide I and II.

3. Precision and reproducibility

(1) Within assay variation (2 samples, 8 replicates assay)

Average C.V. is 2.3%.

(2) Reproducibility (3 samples, 4 replicates assay, 4 days)

Average C.V. is smaller than 3.5%.

Data

Well	A	B
1	976	238
2	969	230
3	965	230
4	1023	235
5	977	231
6	1018	228
7	1038	229
8	995	225
mean	995	231
SD	27.7	4.10
CV (%)	2.78	1.78

pg/ml

Samples/Day	Day 1	Day 2	Day 3	day 4	mean	SD	CV (%)
C	1502	1500	1499	1501	1500	1.12	0.07
D	301	302	301	300	301	0.66	0.22
E	60.9	63.8	62.2	58.8	61.4	2.13	3.46

pg/ml , n=4

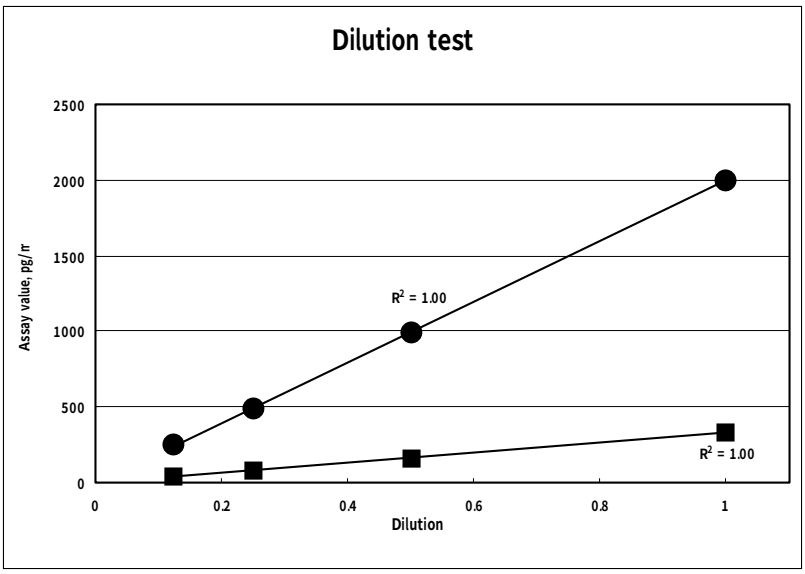
4. Recovery test

Added	Found	Recovered	Rate (%)
0.00	300	-	-
265	551	250	94
398	683	382	96
531	827	527	99

Added	Found	Recovered	Rate (%)
0.00	58.2	-	-
28.9	86.7	28.5	99
38.6	98.2	40.0	104
77.4	139	80.8	104

pg/ml, n=2

5. Dilution test



6. Cross reactivity

Species	Substances	Cross-reactivity (%)
Mouse	C-peptide I	100
	C-peptide II	100
	Insulin	Less than sensitivity
	Pro-Insulin	Less than sensitivity
Rat	C-peptide	90
	Insulin	Less than sensitivity
	Pro-Insulin	Less than sensitivity
Human	C-peptide	95
	Insulin	Less than sensitivity
	Pro-Insulin	Less than sensitivity

Cross reactivity was estimated at 15,000pg/ml of the substances.

[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 not 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]

Six months from production. Expiration date is indicated on the container.

[Unit of package]

96-wells/1 plate

[Product code]

AKRCP-031

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