SHIBAYAGI	Rat Insulin ELISA KIT (S-Type)	Research Reagent

Please, read this instruction carefully before use.

This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of rat insulin with high specificity and high sensitivity using Sandwich assay principle with least influence of co-existing proinsulin.

[Advantage]

(1) Rapid assay (total reaction time: 2 hours 50min.).
(2) A small sample volume (10μl in standard 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-rat insulin-coated plate	96 wells(8x12) / 1 plate
(B)	Standard rat insulin solution (200ng/ml)	50μl / 1 vial
(C)	Buffer solution	60ml/**1** vial
(D)	Biotin-conjugated anti-rat insulin	200μl/ 1 vial
(E)	Peroxidase-conjugated streptavidin	200μl/ 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]

Rat serum or plasma 10μl in the standard procedure.

[Assay range]

0.1 ~ 10ng/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-insulin (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 10ng/ml, then prepare lower standard solutions by a dilution program shown below.

Conc.(ng/ml)	10	5	2.5	1	0.5	0.25	0.1	0
Std. Sol.(μl)	10**	50*	50*	50*	50*	50*	50*	0
Buffer (μl)	190	50	50	75	50	50	75	50

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

4. Assay procedure

(1) Remove the cover sheet of the microplate after getting back to room temperature.
(2) Pipette 100μl of biotin-conjugated anti-insulin solution to all the wells.
(Pre-washing of the plate is not necessary.)
(3) Shake the plate gently on a plate shaker.
(4) Pipette 10μl of sample to sample-assay wells.
(5) Pipette 10μl of standard solution to the wells for preparing a standard curve.
(6) Shake the plate as (3).
(7) Incubate for 2 hour at room temperature (20-25C).
(8) Discard the reaction mixture. Rinse wells by filling the washing buffer and discard 4 times, then strike the plate upside-down onto folded several sheets of paper towel to remove buffer drops remaining in wells.
(9) Pipette 100μl of HRP-conjugated avidin solution to all wells, and shake as (3).
(10) Incubate the plate for 30 minutes at room temperature.
(11) Discard the reaction mixture, and then wash the plate as (8).
(12) Pipette 100μl of chromogenic substrate solution to wells, and shake as (3).
(13) Incubate the plate for 20 minutes at room temperature.
(14) Add 100 μl of the reaction stopper (H) to all wells and shake as (3).
(15) 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

↓

Biotin-conjugated anti-insulin 100μl

↓

Shaking

↓

Standard or sample 10µl

↓

Shaking and reaction for 2 hours at room temp.

↓

Washing 4 times

↓

Peroxidase-avidin conjugate 100µl

↓

Shaking and reaction for 30 mins. at room temp.

↓

Washing 4 times

↓

Chromogenic substrate solution 100µl

↓

Shaking, and reaction for 20 mins. at room temp

↓

Reaction stopper (1M H_2SO_4) 100µl

↓

Shaking and measurement of absorbance
at 450nm(sub. 620nm)

Room temp.: 20~25C

[Calculation of rat insulin concentration]

(1) Prepare a standard curve using semi-logarithmic or logarithmic section paper by plotting absorbance* (Y-axis) against insulin concentration (ng/ml) on X-axis.
*Absorbance at 450nm minus absorbance at 620nm.

(2) Using the standard curve, read the insulin concentration of a sample from its absorbance*, and multiply the assay value by dilution rate if the sample has been diluted. Though the assay range is wide enough, in case the absorbance 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 the use of 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 matters should be centrifuged before assay and use the clear supernatant fluid.

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

2. Storage of assay samples.

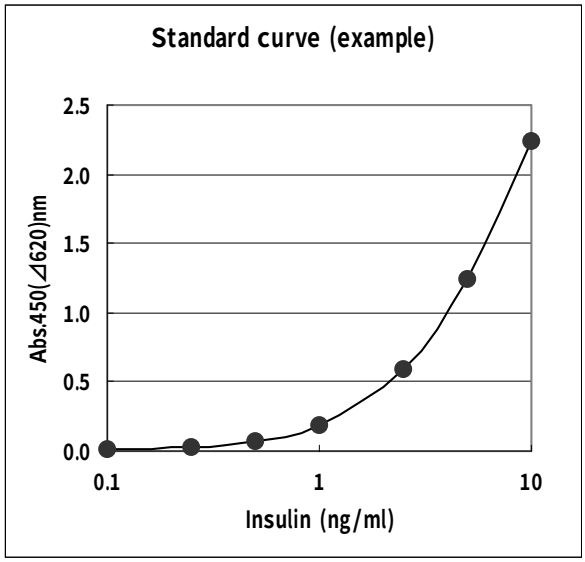
If assay samples have to be stored for a long period, freeze samples and store below –35C. Avoid repeated freezing and thawing.

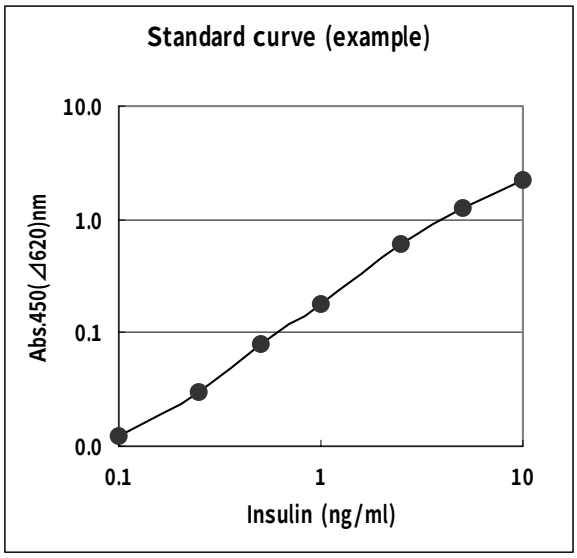
3. Influence of interfering substances

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

This is an ELISA system that captures only insulin. Cross-reactivity to rat proinsulin is less than 5% when 100ng/ml rat proinsulin is added to the system.

See detailed data shown below.

Rat proinsulin addition test（Sample No. G）

Rat proinsulin added	Found as insulin	Effect on insulin assay (%)
0	1.00	-
1.0	1.01	1.0
2.0	1.03	1.5
4.0	1.07	1.8
8.0	1.19	2.4

unit：ng/ml, n=2

Cross-reactivity to insulin-related materials.

Materials tested	Reactivity (%)	Conc. tested
Rat Insulin	100	
Rat C-peptide	Less than lower limit	100ng/ml
Rat Proinsulin	< 5	100ng/ml
Mouse Insulin	102	10ng/ml
Mouse C-peptide	Less than lower limit	100ng/ml
Porcine Insulin	120	10ng/ml
Dog Insulin	Cross-reacted	10ng/ml
Bovine Insulin	Cross-reacted	10ng/ml
Human Insulin	185	10ng/ml
Rabbit Insulin	180	10ng/ml

3. Precision and reproducibility

(1) Within assay variation (3 samples, 8 replicates assay)

Average C.V. is less than 5%.

	Samples		
	A	B	C
1	6.51	2.97	1.11
2	6.39	2.91	1.01
3	5.97	2.89	1.03
4	6.09	2.94	1.02
5	6.01	2.89	1.00
6	5.95	2.84	1.02
7	5.97	2.98	1.02
8	6.29	2.98	1.05
mean.	6.15	2.92	1.03
SD	0.22	0.051	0.034
CV(%)	3.6	1.8	3.3

unit：ng/ml

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

Average C.V. is less than 5%.

Samples	Day 0	Day 1	Day 2	Day 3	mean.	SD	CV(%)
D	5.10	5.03	5.12	5.12	5.09	0.041	0.8
E	0.989	0.965	0.950	0.984	0.972	0.018	1.8
F	0.510	0.513	0.522	0.536	0.520	0.012	2.2

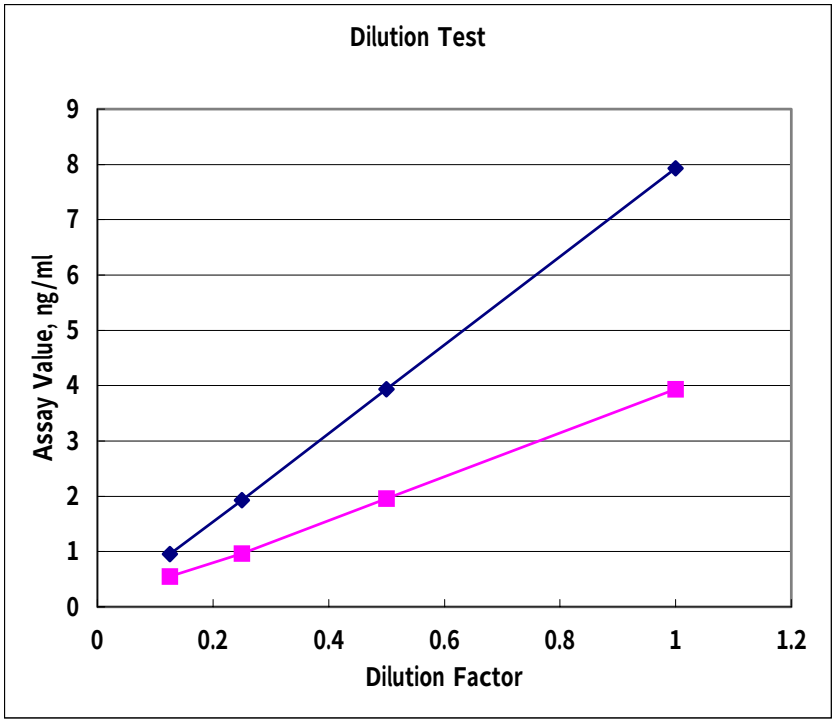
unit：ng/ml, n=3

4. Recovery test

Sample No.G			
Added	Found	Recovered	Recovery(%)
-	1.01	-	-
0.19	1.19	0.18	95
0.39	1.38	0.37	95
0.79	1.76	0.75	95
1.97	2.99	1.98	101
Sample No.H			
Added	Found	Recovered	Recovery(%)
-	4.24	-	-
1.69	5.89	1.65	98

3.39	7.50	3.26	96
5.08	9.10	4.86	96
6.88	10.6	6.37	94

5. Dilution test



[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]

AKRIN-010S

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