SHIBAYAGI	Mouse 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 mouse 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: 3 hours.).
(2) A small sample volume (5µl 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-mouse insulin-coated plate	96 wells (8x12) / 1 plate
(B)	Standard mouse insulin solution (5000pg/ml)	500µl / 1 vial
(C)	Buffer solution	60ml/**1** vial
(D)	Biotin-conjugated anti-mouse 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]

Mouse serum or plasma, 5µl in the standard procedure.

[Assay range]

78 ~ 5000pg/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

Use the original standard solution (B) as the highest standard, then prepare lower standards by serial dilution with the buffer solution as shown below.

Conc.(pg/ml)	5000	2500	1250	625	313	156	78	0
Std. Sol.(μl)	Orig. sol.	Orig. sol. 50	50*	50*	50*	50*	50*	0
Buffer (μl)	0	50	50	50	50	50	50	50

*One rank higher standard solution

4. Assay procedure

Remove the cover sheet of the microplate after getting back to room temperature.

(1) 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.
(2) Pipette 50μl of biotin-conjugated anti-insulin solution to all the wells, and shake the plate on a plate shaker.
(3) Pipette 5 μl of samples to the sample wells.
(4) Pipette 5μl of standard solutions prepared above to the wells for preparing a standard curve.
(5) Shake the plate as (2).
(6) Incubate for 2 hours at room temperature (20-25C).
(7) 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.
(8) Pipette 50μl of HRP-conjugated avidin solution to all wells, and shake as (2).
(9) Incubate the plate for 30 minutes at room temperature.
(10) Discard the reaction mixture, and then wash the plate as (8).
(11) Pipette 50μl of chromogenic substrate solution to wells, and shake as (2).
(12) Incubate the plate for 30 minutes at room temperature.
(13) Add 50 μl of the reaction stopper (H) to all wells and shake as (2).
(14) 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 98 well plate

↓

Washing 4 times

↓

Biotin-conjugated anti-insulin 50μl

↓

Shaking

↓

Standard or sample 5μl

↓

Shaking and reaction for 2 hours at room temp.

↓

Washing 4 times

↓

Peroxidase-avidin conjugate 50μl

↓

Shaking and reaction for 30 mins. at room temp.

↓

Washing 4 times

↓

Chromogenic substrate solution 50μl

↓

Shaking, and reaction for 30 mins. 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 mouse insulin concentration]

(1) Prepare a standard curve using semi-logarithmic or dual logarithmic section paper by plotting absorbance* (Y-axis) against insulin concentration (pg/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 previously. 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.

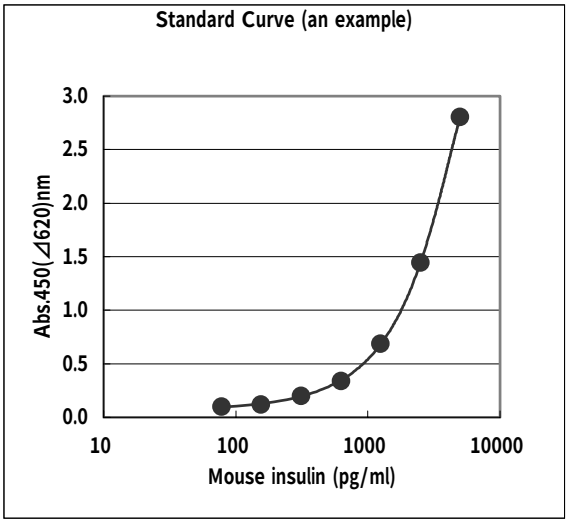
If assay samples have to be stored for a long period, freeze samples and store below –35C. Avoid repeated freezing and thawing.

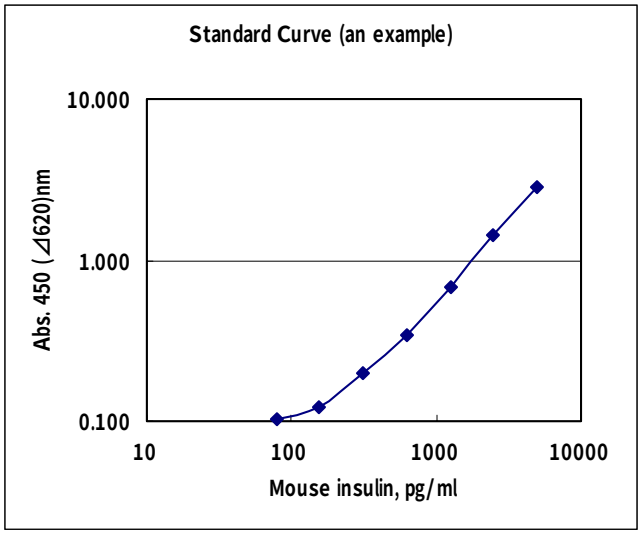
3. Influence of interfering substances

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 mouse proinsulin is less than 5% when 50ng/ml mouse proinsulin is added to the system.

See detailed data shown below.

Mouse proinsulin addition test（Sample No. I）

Proinsulin added	Assay value as insulin	Crossreactivity of proinsulin(%)
0	106.0	-
100	106.9	0.9
500	112.5	1.3
5000	286.0	3.6

unit：pg/ml, n=2

Cross-reactivity to insulin-related substances.

Related substance	Reactivity(%)	Note
Mouse Insulin	100	
Mouse C-peptide	<lower limit	at 5 0ng/ml

Mouse Proinsulin	< 5	at 50ng/ml
Rat Insulin	98	at 10ng/ml
Rat C-peptide	<lower limit	at 50ng/ml
Porcine Insulin	118	at 10ng/ml
Dog Insulin	Cross reacted	at 10ng/ml
Bovine Insulin	Cross reacted	at 10ng/ml
Human Insulin	185	at 10ng/ml
Rabbit Insulin	180	at 10ng/ml

3. Precision and reproducibility

(1) Within assay variation (3 samples, 8 replicates assay) Unit: pg/ml

No.	Sample A	Sample B
1	2488	491
2	2477	470
3	2410	457
4	2434	465
5	2433	459
6	2342	442
7	2358	459
8	2390	495
Mean.	2417	467
SD	52.3	17.8
CV (%)	2.2	3.8

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

Sample No.	Day 0	Day 1	Day 2	Day 3	Day4	SD	CV (%)
C	166	156	153	153	157	6.16	3.9
D	628	625	624	624	625	1.89	0.30
E	2505	2409	2427	2549	2473	65.86	2.7

Unit: pg/ml

4. Recovery test

Sample F

Added	Found	Recovered	Recovery (%)
0	1315	-	-
324	1632	317	97.8
630	1935	620	98.4
889	2172	857	96.4
1305	2625	1310	100

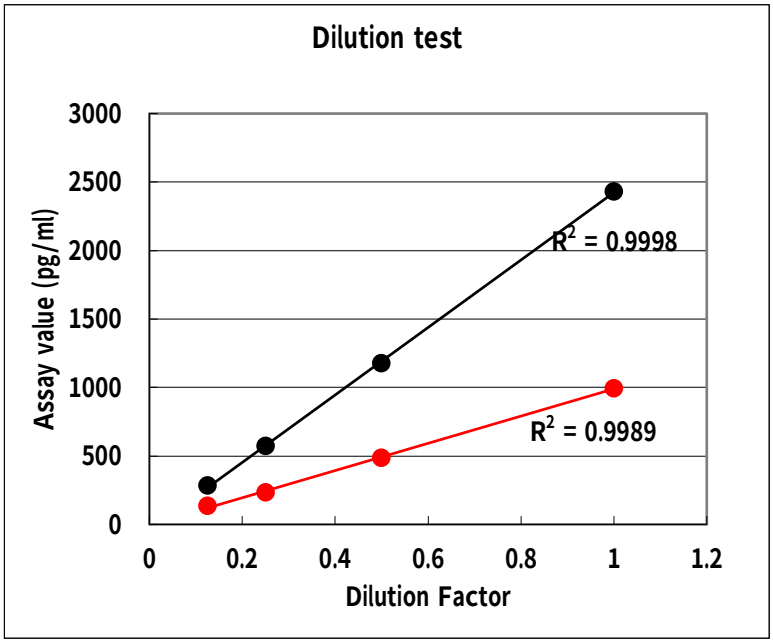
Sample G

Added	Found	Recovered	Recovery (%)
0	512	-	-

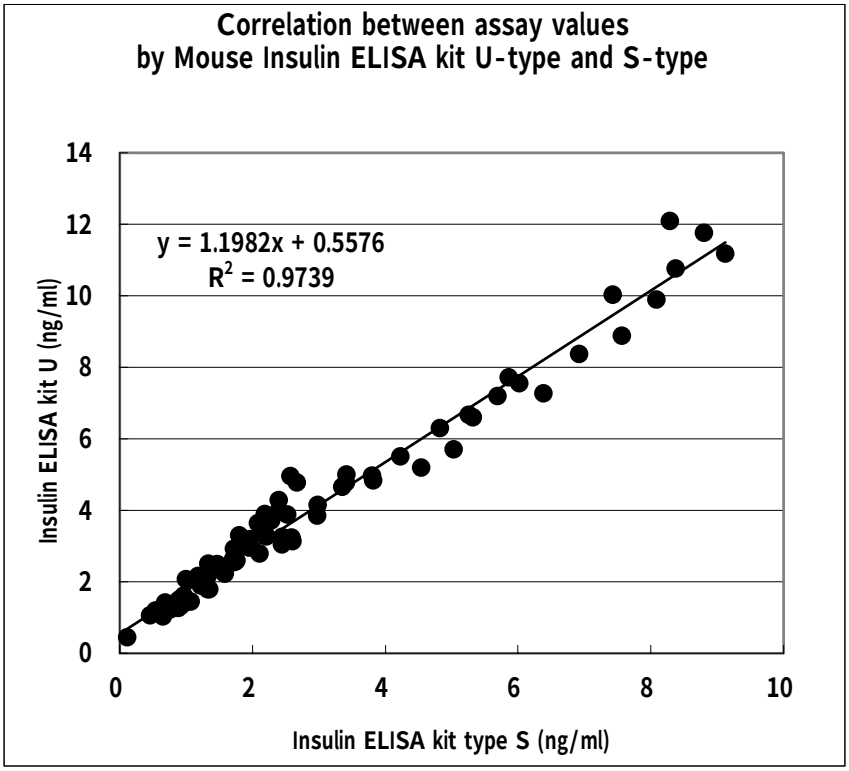
426	925	413	96.9
559	1073	561	100
662	100	665	101
745	1270	758	102

Unit: pg/ml n=3

5. Dilution test



6. Correlation of assay values between AKRIN 011T and AKRIN 011S



Samples: 75 mouse sera, Unit: ng/ml

[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-011S

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