Insulin (mouse) ELISA Kit
(S-Type)

For Product: Cat. No. S-9106

For research use only

NOTE: Please read the "Statements and Precautions" section prior to working with the kit.

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This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of mouse insulin with high specificity and high sensitivity using a Sandwich format to minimize interference by co-existing proinsulin.

I. Advantages:

(1) Rapid assay (total reaction time: 3 hours.).
(2) Small sample volume (5 µl 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.

II. Components:

	Reagents	Amounts
(A)	Anti-mouse insulin-coated plate	96 wells (8x12) / 1 plate
(B)	Standard mouse insulin solution (5000 pg/ml)	500 µl / 1 vial
(C)	Buffer solution	60 ml/1 vial
(D)	Biotin-conjugated anti-mouse insulin	200 µl/ 1 vial
(E)	Peroxidase (HRP)-conjugated streptavidin	200 µl/ 1 vial
(F)	Chromogenic substrate reagent (TMB)	12 ml/ 1 vial
(H)	Stop Solution (1M H_2SO_4)	12 ml/ 1 vial
(I)	Concentrated wash buffer (10x)	100 ml/ 1 bottle

III. Assay sample type:

Mouse serum or plasma (5 µl in the standard procedure).

IV. Assay range:

78 ~ 5000 pg/ml

V. Assay Procedure:

1. Materials 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 wash bottle with nozzle).
(3) Microplate reader

2. Preparation of reagents

(1) Wash buffer: Dilute the concentrated wash buffer (I) 1:10 with purified water.
(2) Biotin-conjugated anti-insulin (D): Dilute 1:100 with the buffer solution (C).
(3) HRP-conjugated streptavidin (E): Dilute 1:100 with the buffer solution (C).
(4) Other reagents are used as provided.
(5) All the reagent solutions should be equilibrated to room temperature (20-25 ^{o}C) prior to use.

3. Preparation of standard solutions (example)

Use the original standard solution (B) as the highest standard, and then prepare lower standards concentrations 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

*Previous standard solution

4. Assay procedure

Remove the cover sheet of the microplate after equilibrating to room temperature.

1. Rinse wells by filling with wash buffer and discarding 4 times, then strike the plate upside-down onto several sheets of folded paper towels to remove remaining buffer.

2. Pipette 50 µl of biotin-conjugated anti-insulin solution into all the wells, and shake the plate on a plate shaker.
3. Pipette 5 µl of samples into the sample wells.
4. Pipette 5 µl of standard solutions prepared above into the wells for preparing a standard curve.
5. Shake the plate as in step (2).
6. Incubate for 2 hours at room temperature (20-25 ^{O}C).
7. Discard the reaction mixture and then wash wells as described in (1.).
8. Pipette 50 µl of HRP-conjugated streptavidin solution into all wells, and shake as in step (2).
9. Incubate the plate for 30 minutes at room temperature.
10. Discard the reaction mixture, and then wash the plate as described in (1).
11. Pipette 50 µl of chromogenic substrate solution into wells, and shake as in step (2).
12. Incubate the plate for 30 minutes at room temperature.
13. Add 50 µl of the stop solution (H) to all wells and shake as in step (2).
14. Measure the absorbance of each well at 450 nm (620nm if read before adding Stop Solution) by a plate reader within 30 minutes.

VI. Summary of Assay Procedure:

Antibody-coated 98 well plate

↓

Wash 4 times

↓

Biotin-conjugated anti-insulin 50 µl

↓

Shaking

↓

Standard or sample 5 µl

↓

Shaking and reaction for 2 hours at room temp.

↓

Wash 4 times

↓

Peroxidase-avidin conjugate 50 µl

↓

Shaking and reaction for 30 mins. at room temp.

↓

Wash 4 times

↓

Chromogenic substrate solution 50 µl

↓

Shaking, and reaction for 30 mins. at room temp

↓

Reaction stopper (1M H_2SO_4) 50 µl

↓

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

Room temp. : 20-25 ^{o}C

VII. Calculation of Mouse Insulin concentration

(1) Prepare a standard curve using a semi-log plot, by plotting absorbance (Y-axis) against log of insulin concentration (X-axis, ng/ml).
*Absorbance at 450nm minus absorbance at 620nm.

(2) Using the standard curve, determine the insulin concentration of a sample from its measured absorbance.*, If the sample plasma is diluted, multiply the concentration by sample dilution factor to obtain the insulin concentration of the original sample. Though the assay range is very wide, if 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.

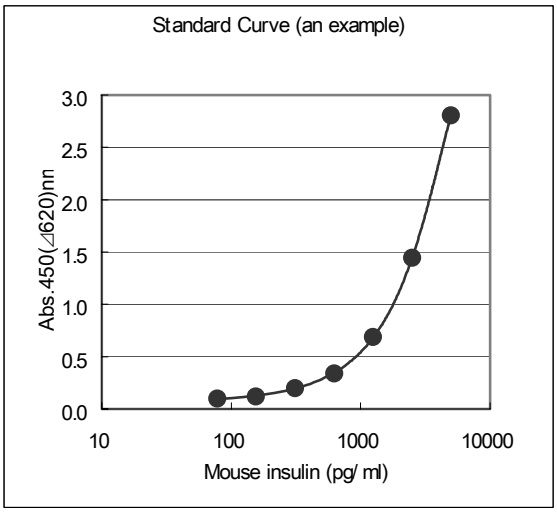
* We recommend the use of 3rd order regression curve or 4-parameter log-logistical model.

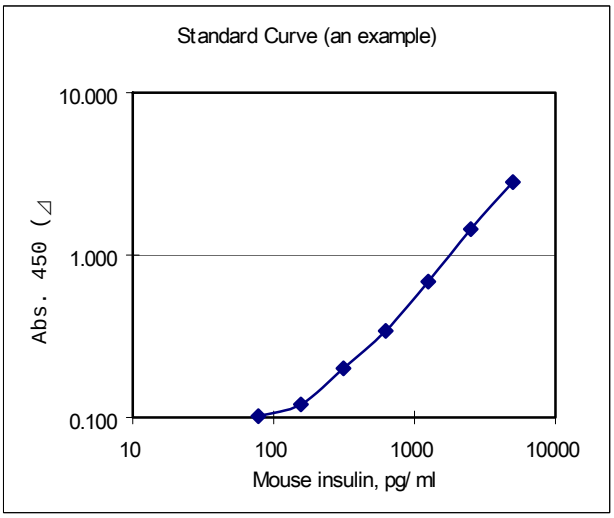
VIII. Important notice regarding sample treatments

1. Treatment of assay samples
   (a) Use serum or plasma samples obtained by standard methods.
   (b) Turbid samples or those containing insoluble matter should be centrifuged and the clarified supernatant should be used in the assay.
   (c) Measure the samples as soon as possible after sampling.
2. Storage of assay samples.
   If assay samples must be stored for a long period, freeze samples and store below -35°C. Avoid repeated freezing and thawing.
3. Influence of interfering substances
   If the presence of interfering substances is suspected, examine by a dilution test using more than 2 points.

IX. Assay range and assay validation

1. Example standard curves





2. Specificity
   This is an ELISA system that measures only insulin. Cross-reactivity to mouse proinsulin is less than 5% when 50 ng/ml of 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 50 ng/ml
Mouse Proinsulin	< 5	at 50 ng/ml
Rat Insulin	98	at 10 ng/ml
Rat C-peptide	<lower limit	at 50 ng/ml
Porcine Insulin	118	at 10 ng/ml
Dog Insulin	Cross reacted	at 10 ng/ml
Bovine Insulin	Cross reacted	at 10 ng/ml
Human Insulin	185	at 10 ng/ml
Rabbit Insulin	180	at 10 ng/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

X. Statements and precautions

(1) The reagents included in this assay kit should be used for research purposes only.

(2) Optimally, the reagent solutions of the kit should be used immediately after dilution. Otherwise, keep them in the dark at 2-8°C , and use them within 3 days.
(3) The reagents were prepared to give accurate results with the components provided in the kit. Do not combine the reagents from kits of other lot numbers. Even when the lot number is the same, do not mix the reagents with those that have been stored for some period.
(4) Pipetting and dilution of the reagent solutions should be done accurately because these steps influence the assay precision.
(5) Do not dry the assay plate to avoid denaturation of the coated antibody.
(6) The reaction time should be counted from the onset of reagent pipetting.
(7) Prepare a new standard curve for every new assay.
(8) Dilution of the assay sample must be carried out using the buffer solution included in the kit.
(9) Storage conditions for the kit or its components should be strictly observed.
(10) Be careful not to allow the reagent solutions of the kit to touch the skin, mucus membranes, or eyes. Be especially careful of the stop solution because it contains sulfuric acid.
(11) The HRP-conjugated reagent solution, the chromogenic substrate solution, and the stop solution should not be allowed to contact any metal.
(12) In treating assay samples of animal origin, be careful for possible biohazards.
(13) As the antibody-coated plate is a module modular type of 8wells x 12 rows, each row can be separated by a cutter and used independently.

XI. Storage condition:
Store the kit at 2-8^{o}C. **Do not freeze**

XII. Term of validity: Six months from production. Expiration date is indicated on the container.

XIII. Unit of package: 96-wells/1 plate

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