	Mouse Insulin ELISA KIT (H－Type)	For in-vitro laboratory use only!

Please, read this instruction carefully before use.

This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of mouse linsulin with a proper sensitivity for diabetic model animals using Sandwich assay principle.

[Advantage]

(1) Rapid assay (total reaction time: 3 hours).
(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)	Antibody-coated plate	96 wells(8x12) / 1 plate
(B)	Standard mouse insulin solution (200ng/ml)	300μl / 1 vial
(C)	Buffer solution	60ml/ 1 vial
(D)	Biotin-conjugated anti-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 : 10μl/well
Cultured tissue, cells and media

[Assay range]

0.5 ~ 100 ng/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

(An example) Prepare the highest standard solution by 1:1 dilution of the original standard solution, then prepare lower standards by a dilution program shown below.
(You can use other mode of dilution for a set of standard solutions.)

Std. Conc. (ng/ml)	100	50	25	10	5.0	1.0	0.5	0
Std. Sol.(μl)	100**	100*	100*	100*	100*	100*	100*	0
Buffer (μl)	100	100	100	150	100	400	100	100

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

4. Assay procedure

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

(1) Rinse the antibody-coated wells (A) by filling the washing buffer and discard 4 times, then strike the plate upside-down onto folded several sheets of paper towel, and remove the excess buffer.
(2) Pipette 100μl of biotin-conjugated anti-insulin (D) to all the wells, then shake gently on a plate shaker.
(3) Pipette 10μl of sample into the wells for samples.
(4) Pipette 10μl of the standard solution to the wells for preparing a standard curve.
(5) Shake the plate gently on a plate shaker.
(6) Incubate the plate for 2 hours at room temperature.
(7) Discard the reaction mixture, and then wash the plate 4 times as described in (1), and remove excess washing buffer remaining in the wells as (1).
(8) Pipette 100μl of HRP-conjugated avidin solution to all wells, and shake as (5).
(9) Incubate for 30 minute at room temperature.
(10) Discard the reaction mixture, and then wash the plate 4 times as (2), and remove excess washing buffer
(11) Pipette 100μl of chromogenic substrate solution to wells, and shake as (5).
(12) Let the plate stand for 30 minutes at room temperature.
(13) Add 100 μl of the reaction stopper (H) to all wells and shake.
(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 96 well plate

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Washing 4 times

↓

Biotin-conjugated anti-insulin antibody 100µl

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Sample or Standard 10µl

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Shaking and reaction for 2 hour at 20~25C

↓

Washing 4 times

↓

Peroxidase-conjugated avidin 100µl

↓

Shaking, and reaction for 30 min at 20~25C

↓

Washing 4 times

↓

Chromogenic substrate solution 100µl

↓

Shaking, and reaction for 30 min. at 20~25C

↓

Reaction stopper (1M H_2SO_4) 100µl

↓

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

[Calculation of insulin concentration]

(1) Prepare a standard curve using normal or semi-logarithmic or bi-logarithmic section paper by plotting absorbance* (Y-axis) against standard concentration (ng/ml) on X-axis. For the manual reading from the standard curve, we recommend the use of bi-logarithmic section paper.

*Absorbance at 450nm minus absorbance at 620nm.

(2) Read insulin concentration of a sample from its absorbance. Though the assay range is wide enough, in case the absorbances 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. If you use logarithm transformation for insulin concentration and absorbance, the fitness of the 3rd order regression curve will be improved.

[Important notice in the treatments]

1. Treatment of assay samples

(1) Use serum or plasma samples obtained by ordinary standard method.
We recommend heparin for obtaining plasma samples.

(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.

(4) It would be also convenient to dilute the assay samples first in test tubes, and pipette 50μl of the diluted sample to a well.

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]

A model standard curve