SHIBAYAGI	Rat Insulin ELISA Kit (TMB)	Research Reagent

Please, read this instruction carefully before use.

[Advantage]

(1) Rat Insulin Kit is a high speed EIA. (for 3-4 hours).

(2) Rat Insulin Kit can measure in serum or plasma* samples of very small volume(10µl).

(3) Rat Insulin Kit ensures simple assay procedures.

We recommend the use of heparin to obtain plasma.

[Reagents]

A:	Anti-Insulin-coated plate	96well(8x12)	x1
B:	Standard Rat insulin solution (200ng/ml)	25µl	x1
C:	Buffer solution	60ml	x1
D:	Biotin-conjugated anti-insulin	10µl	x1
E:	HRP-conjugated streptavidin	20µl	x1
F:	Chromogenic substrate reagent (TMB)	12ml	x1
H:	Reaction stopper (1M H_2SO_4)	12ml	x1
I:	Concentrated washing buffer (10x)	100ml	x1

[Preparation of reagents]

*Reagent solutions should be used immediately after preparation.

- Standard solutions of insulin
  Prepare standard solutions by dilution as shown by an example below.
- Biotin-conjugated anti-insulin
  Prepare by dilution of (D) with the buffer(C) to 1:4000.
- HRP-conjugated streptavidin
  Prepare by dilution of (E) with the buffer(C) to 1:2000.
- Chromogenic substrate solution (F)
  Use the original solution without dilution.

' Reaction stopper (H)
Use the original solution without dilution.
' Washing buffer
Prepare by dilution of concentrated bffer (I) with purified water to 1:10.

Preparation of standard insulin solutions (an example)

Insulin conc. (pg/ml)	10000	5000	2500	1250	625	312	156	0
Standard insulin (μl)	10*	100**	100**	100**	100**	100**	100**	0
Buffer solution (μl)	190	100	100	100	100	100	100	100

*Original stnadard insulin solution **One rank higher standard solution

As an example shown above, first prepare 10000pg/ml from attached original standard solution, and then, by serial dilution, prepare 5000pg/ml, 2500pg/ml, and so on.

For the zero standard, use buffer solution alone.

Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.

[Assay Procedure]

01) Rinse the anti-insulin coated plate(A) 4 times with washing buffer(I).

02) Pipette 100μl of Biotin-conjugated anti-insulin(D) into each well, and shake.

03) Pipette 10μl sample or standard Insulin solution(B) into each well and shake.

04) Incubate for 2hours at room temperature.(20-25C).

05) Rinse the plate 4 times with washing buffer(I).

06) Pipette 100μl of HRP-conjugated streptavidin solution(E) into each well and shake.

07) Incubate for 30 minutes at room temperature. (20-25C).

08) Rinse the plate 4 times with washing buffer(I).

09) Pipette 100μl of chromoqenic substrate reagent(F) into each well and shake.

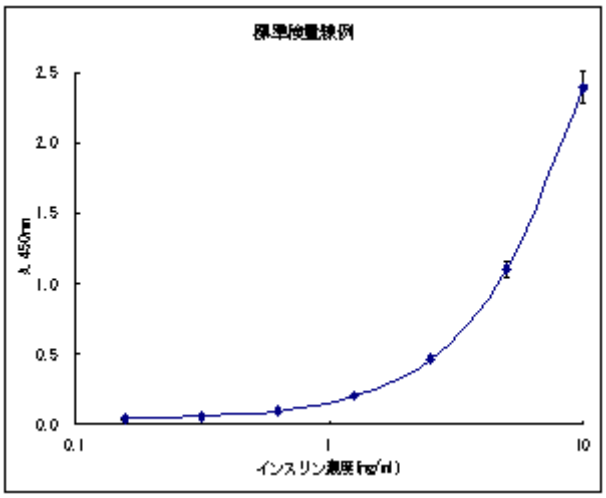
10) Incubate for 30 minutes at room temperature (20-25C).

11) Pipette 100ml of Reaction stopper(H) into each well and shake.

12) Measure each well's absorbance at 450 nm by the plate reader within 30 minutes.

[Calculation of Insulin Concentration]

1. Using hemi-logarithmic section paper, prepare a standard curve by plotting absorbance(Y-axis) against logarithm of insulin concentration (X-axis, ng/ml).
2. Using the standard curve, read the insulin concentration of a sample from their absorbance.
3. In case sample plasma is diluted, then multiply the concentration by sample dilution rate to obtain the insulin concentration of the original sample.



[Summary of Assay Procedure]

Antibody-coated plate
Washing
| +Biotin-conjugated anti-insulin 100µl, adn shaking
| +sample or standard Insulin solution 10µl
Shaking , Reaction at room temp.(20 - 25C) for 2hr
|
Washing
| +HRP-avidin; 100µl
Shaking , Reaction at room temp.(20 - 25C) for 30mins
Washing
| + Chromogenic substrate solutin; 100µl
Shaking , Reaction at room temp.(20 - 25C) for 30mins
| + Reaction stopper; 100µl
Shaking , Measurement of Absorbance(450nm)

[Precision and Reproducibility]

Precision (Within assay variation) : Average C.V.: 2.06 %

Reproducibility (Between assay variation) : Average C.V.: 2.87 %)

[Storage Condition]

2-8C, in a dark place,. Do not freeze.

[Statements and Precautions]

01. This assay kit or its components should be used only for research works.
02. The reagent solutions of the kit should be used principally immediately after dilution. Otherwise, keep them in a dark place at 2-8℃, and use them within 5 days.
03. 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 are preserved for some period.
04. Pipetting and dilution of the reagent solutions should be made accurately because these steps influence the assay precision.
05. Do not dry the assay plate to avoid denaturation of the coated antibody or antigen.
06. The reaction time should be counted from the onset of reagent pipetting.
07. Prepare the standard curve in every assay. (For kits with standard solution.)
08. Dilution of the assay sample must be carried out using the buffer solution attached to the kit.
09. Preservation condition for the kit or its components should be strictly kept.
10. ***Be careful not to allow the reagent solutions of the kit to contact with skin, mucus and eyes (wearing glasses for protection is recommended). Especially treat the stopping solution very carefully because it contains sulfuric acid.***
11. HRP-conjugated reagent solution, chromogenic substrate solution, and reaction stopper should be avoided from contacting with any metal.
12. ***In treating assay samples of animal origin, be careful for possible biohazards.***

Please, refer "All about Shibayagi's insulin kits" for further information.

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