

**Cat. No.: RSHAKRIN011TR**

**Please, read this instruction carefully before use.**

### **[Advantage]**

- (1) Mouse Insulin Kit is a high speed EIA. (for 3-4 hours).
- (2) Mouse Insulin Kit can measure in serum or plasma\* samples of very small volume(10 µl).
- (3) Mouse Insulin Kit ensures simple assay procedures.

\*We recommend the use of heparin for obtaining plasma.

### **[Reagents]**

A: Anti-Insulin-coated plate	96well(8x12)	x1
B: Standard mouse insulin solution (200ng/ml)	25µl	x1
C: Dilution Buffer solution	60ml	x1
D: Biotin-conjugated anti-insulin	10 µl	x1
E: HRP-conjugated streptavidin	20 µl	x1
F: Chromogenic substrate reagent (TMB)	12ml	x1
H: Reaction Stop Solution (1M H <sub>2</sub> SO <sub>4</sub> )	12ml	x1
I: Concentrated Wash 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 Stop Solution (H)

Use the original solution without dilution.

· Wash Buffer

Prepare by dilution buffer of concentrated buffer (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 ( $\mu$ l)	10*	100**	100**	100**	100**	100**	100**	0
Buffer solution ( $\mu$ l)	190	100	100	100	100	100	100	100

\*Original standard 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.

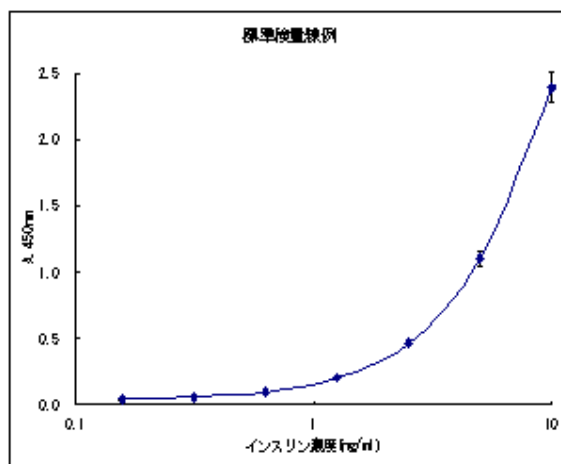
Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.

### [Assay Procedure]

- 01) Rinse the anti-insulin coated wells (A) 4 times by filling with wells with 350  $\mu$ l of Wash Buffer(I). Discard the buffer. Remove residual buffer in the wells by striking the plate upside-down onto several sheets of folded paper towels.
- 02) Pipette 100  $\mu$ l of Biotin-conjugated anti-insulin(D) into each well, and shake on a plate shaker at 800-1000 rpm for ~ 5-10s.
- 03) Pipette 10  $\mu$ l sample or standard Insulin solution(B) into each well and shake as in (2).
- 04) Incubate for 2 hours at room temperature.(20-25C).
- 05) Rinse the plate 4 times with Wash Buffer(I) as in (1).
- 06) Pipette 100  $\mu$ l of HRP-conjugated streptavidin solution(E) into each well and shake as in (2).
- 07) Incubate for 30 minutes at room temperature. (20-25C).
- 08) Rinse the plate 4 times with Wash Buffer (I) as in (1).
- 09) Pipette 100  $\mu$ l of chromogenic substrate reagent (F) into each well and shake as in (2).
- 10) Incubate for 30 minutes at room temperature (20-25C). Avoid exposing the plate to direct sunlight. Covering the plate with aluminum foil is recommended.
- 11) Pipette 100  $\mu$ l of Reaction Stop Solution (H) into each well and shake as in (2).
- 12) Measure each well's absorbance at 450 nm in a plate reader within 30 minutes.

## [Calculation of Insulin Concentration]

1. Using semi-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

Wash

- ↓ +Biotin-conjugated anti-insulin 100  $\mu$ l, and shaking  
+sample or standard Insulin solution 10  $\mu$ l

Shake & Incubate at room temp.(20 - 25C) for 2hr

↓

Wash

- ↓ +HRP-avidin; 100  $\mu$ l

Shake & Incubate at room temp.(20 - 25C) for 30mins

Wash

- ↓ + Chromogenic substrate solutin; 100  $\mu$ l

Shake & Incubate at room temp.(20 - 25C) for 30mins

↓

- + Reaction Stop Solution; 100  $\mu$ l

Shake & Measure Absorbance(450nm)

## [Precision and Reproducibility]

Precision (Within assay variation) : Average C.V.: 1.98 %

Reproducibility (Between assay variation) : Average C.V.: 3.91 % )

## [Storage Condition]

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

### **[Statements and Precautions]**

- 01.** This assay kit or its components are intended for research use only.
- 02.** The reagent solutions of the kit should be used immediately after dilution. However, if necessary, keep them in a dark place at 2-8°C, and use 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 membranes 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 Stop Solution should not be allowed contact 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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