

Cat. No.: RSHAKRIN013R

Please, read this instruction carefully before use.

## 1. ADVANTAGE

1. Porcine Insulin Kit is a high speed EIA. (for 3-4 hours).
2. Porcine Insulin Kit can measure in samples of very small volume (10 $\mu$ l).
3. Porcine Insulin Kit ensures simple assay procedures.  
(Use of heparin is recommended for preparation of plasma samples.)

## 2. REAGENTS

|    |   |              |    |
|----|---|--------------|----|
| A: | Anti-Insulin-coated plate                             | 96well(8x12) | x1 |
| B: | Standard Porcine insulin solution (240ng/ml)          | 25 $\mu$ l   | x1 |
| C: | Buffer solution                                       | 60ml         | x1 |
| D: | Biotin-conjugated anti-insulin                        | 10 $\mu$ l   | x1 |
| E: | HRP-conjugated streptavidin                           | 20 $\mu$ l   | x1 |
| F: | Chromogenic substrate reagent (TMB)                   | 12ml         | x1 |
| H: | Reaction stopper (1M H <sub>2</sub> SO <sub>4</sub> ) | 12ml         | x1 |
| I: | Concentrated washing buffer (10x)                     | 100ml        | x1 |

### 3. PREPARATION OF REAGENT SOLUTIONS

Reagent solutions should be used immediately after preparation.

1. Biotin-conjugated anti-insulin: Dilute the original solution to 1:4000 with the buffer solution.
2. HRP-conjugated streptavidin solution: Dilute the original solution to 1:2000 with the buffer.
3. Chromogenic substrate reagent (TMB): Use the original solution as it is.
4. Concentrated washing buffer: Getting the original solution back to room temperature (20-25°C), dilute it to 1:10 with purified water.
5. Porcine insulin standard solution: Prepare the standard solutions by serial dilution as shown below.

|  |       |       |       |       |       |       |       |     |
|--|-------|-------|-------|-------|-------|-------|-------|-----|
| Insulin conc.(pg/ml)                                     | 12000 | 6000  | 3000  | 1500  | 750   | 375   | 188   | 0   |
| Original std. solution(B)* or higher std. solution**(μl) | 10*   | 100** | 100** | 100** | 100** | 100** | 100** | 0   |
| Buffer solution(μl)                                      | 190   | 100   | 100   | 100   | 100   | 100   | 100   | 100 |

First, prepare 12000pg/ml std. solution by diluting the original std. solution (B), then prepare lower std. solutions by serial dilution.

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

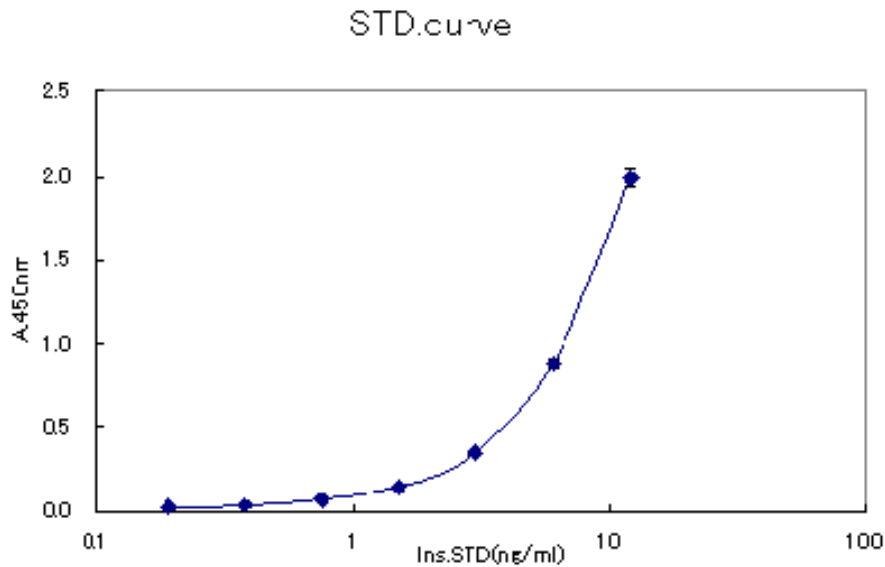
#### 4. ASSAY PROCEDURE

1. Rinse the anti-insulin coated plate (A) 4 times with washing buffer(I).
2. Pipette 100 $\mu$ l of Biotin-conjugated anti-insulin (D) into each well.
3. Pipette 10 $\mu$ l sample or standard Insulin solution (B) into each well and shake.
4. Incubate for 2hours at room temperature. (20-25°C).
5. Rinse the plate 4 times with washing buffer (I).
6. Pipette 100 $\mu$ l of HRP-conjugated streptavidin solution (E) into each well and shake.
7. Incubate for 30 minutes at room temperature. (20-25°C).
8. Rinse the plate 4 times with washing buffer (I).
9. Pipette 100 $\mu$ l of chromogenic substrate reagent (F) into each well and shake.
10. Incubate for 30 minutes at room temperature (20-25°C).
11. Pipette 100 $\mu$ l 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.

#### 5. CALCULATION OF INSULIN CONCENTRATION

1. 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

## 6. AN EXAMPLE OF STANDARD CURVE



## 7. SUMMARY OF ASSAY PROCEDURE

Antibody-coated plate

Washing

+Biotin-conjugated anti-insulin 100 $\mu$ l

+sample or standard Insulin solution 10 $\mu$ l

Shaking , Reaction at room temp.(20 – 25°C) for 2hr

Washing

+HRP-avidin; 100 $\mu$ l

Shaking , Reaction at room temp.(20 – 25°C) for 30mins

Washing

+ Chromogenic substrate solutin; 100 $\mu$ l

+ Reaction stopper; 100 $\mu$ l

Shaking , Measurement of Absorbance(450nm)

## 8. STATEMENTS AND PRECAUTIONS

1. This assay kit or its components should be used only for research works.
2. The reagent solutions of the kit should be used principally immediately after dilution. Otherwise, keep them in a dark place at 2-8°C ,and use them within 5 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 are 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 or antigen.
6. The reaction time should be counted from the onset of reagent pipetting.
7. Prepare the standard curve in every assay. (For kits with standard solution.)
8. Dilution of the assay sample must be carried out using the buffer solution attached to the kit.
9. 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.

## 9. ASSAY PERFORMANCE OF THE KIT

### Accuracy of the kit n=5

| Mean (ng/ml) | S.D.   | CV(%) |
|--------------|--------|-------|
| 0.991        | 0.0321 | 3.24  |
| 0.482        | 0.0175 | 3.63  |
| 0.201        | 0.0099 | 4.93  |

### Reproducibility n=5

| Mean (ng/ml) | S.D.   | CV(%) |
|--------------|--------|-------|
| 1.425        | 0.0562 | 3.87  |
| 0.901        | 0.0321 | 3.56  |
| 0.346        | 0.0162 | 4.68  |

### Recovery test

| Trial | Added(ng/ml) | Assay value (ng/ml) | Found (ng/ml) | Recovery(%) |
|-------|--------------|---------------------|---------------|-------------|
| 1     | 0            | 0.343               | *             | *           |
|       | 0.25         | 0.576               | 0.233         | 93.2        |
|       | 0.5          | 0.802               | 0.459         | 91.7        |
|       | 1            | 1.295               | 0.952         | 95.2        |
|       | 2            | 2.301               | 1.958         | 97.9        |
| 2     | 0            | 0.202               | *             | *           |
|       | 0.25         | 0.437               | 0.235         | 93.8        |
|       | 0.5          | 0.666               | 0.464         | 92.8        |
|       | 1            | 1.201               | 0.999         | 99.9        |
|       | 2            | 2.240               | 2.038         | 101.9       |

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