Version 24-06/24

User's Manual

Equine Insulin ELISA Kit

REF **DEIA-JY2396**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This Insulin ELISA kit is used for the non-radioactive determination of insulin in serum and plasma.

Principles of Testing

This assay is a sandwich ELISA based on two monoclonal antibodies against separate antigenic determinants on the insulin molecule. The microplate is coated with a monoclonal antibody. Standards and samples are added into the wells and react with a monoclonal antibody conjugated to horseradish peroxidase (HRP) enzyme. After washing, TMB substrate is added to the wells and color develops in proportion to the amount of insulin bound. The assay is stopped and the intensity of the color is measured at 450 nm. The amount of insulin in the sample can be calculated from a standard curve.

Reagents And Materials Provided

1. Microplate 96 wells
2. Wash buffer (10×) 20 ml
3. Assay buffer 9 ml
4. Detection antibody solution (100×) 0.09 ml
5. Insulin standard (0 μIU/ml) 1 ml
6. Insulin standard (3 μIU/ml) 0.2 ml
7. Insulin standard (7 μIU/ml) 0.2 ml
8. Insulin standard (16 μIU/ml) 0.2 ml
9. Insulin standard (40 μIU/ml) 0.2 ml
10. Insulin standard (100 μIU/ml) 0.2 ml
11. Substrate solution 12ml
12. Stop solution 12ml
13. Plate sealer 1P

Materials Required But Not Supplied

1. Pipettes and pipette tips.
2. Distilled water or deionized water.
3. Volumetric containers and pipettes for reagent preparation.
4. Paper towels or absorbent paper.
5. Multi-channel micropipettes or automated microplate washer.
6. Microplate shaker capable of 600 rpm.
7. Microplate reader capable of reading absorbance at 450 nm.

Storage

The kit should be stored at 2-8°C upon receipt. Remove any unused antibody-coated strips from the micro-plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2- 8°C for up to one month.

Specimen Collection And Preparation

If a sample has a greater concentration of insulin than the highest standard, the sample should be diluted with 0 ng/ml insulin standard solution and the assay should be repeated.

Reagent Preparation

1. 1× Wash buffer

Prepare 1× Wash buffer by mixing the 10× Wash buffer (20 ml) with 180 ml of distilled water or deionized water. The 1× Wash buffer may be stored at 2-8°C for up to one month.

2. 1× Detection antibody solution

Prepare 1× Detection antibody solution by dilution of the 100× Detection antibody solution in Assay buffer, mix well. 70 µl of the 1× Detection antibody solution is required per well.

Assay Procedure

Note: All reagents and micro-plate strips should be equilibrated to room temperature prior to use. A standard curve must be performed with each assay. It is recommended that all standards and samples should be run in duplicate.

1. Add 70 µl of 1x Detection antibody solution to each well.
2. Add 20 µl of each standard or sample to its respective well.
3. Cover the plate with a plate sealer. Incubate at room temperature for 1 hour, shaking the plate at 600 rpm on a horizontal micro-plate shaker.

(* Alternative incubation step in the absence of shaker: gently taptheplateframe for a few seconds to ensure thorough mixing, incubateat room temperature for 1.5 hours.)

4. Discard well contents and remove any remaining solution by inverting and tapping the plate on a clean paper towel. Add 300 µl of 1× Wash buffer to each well. Incubate at room temperature for 30 seconds.
5. Discard the 1× Wash buffer and tap the plate on a clean paper towel toremove residual wash buffer. Repeat the wash step for a total 4 washes.
6. Add 100 µl of Substrate solution to each well, incubate at room temperaturefor 15 minutes. Protect from light.
7. Add 100 µl of Stop solution to each well, gently tap the plate frame for afew seconds to ensure thorough mixing. Measure absorbance of each well at 450 nm immediately.

Summary of the assay procedure

1. Add 70 µl of 1× Detection antibody solution to each well.

2. Add 20 µl of standard or sample to its respective well.
3. Incubate at room temperature for 1 hour (600 rpm).
4. Wash each well 4 times.
5. Add 100 µl of Substrate solution to each well.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl of Stop solution to each well.
8. Measure absorbance of each well at 450 nm.
9. Calculation

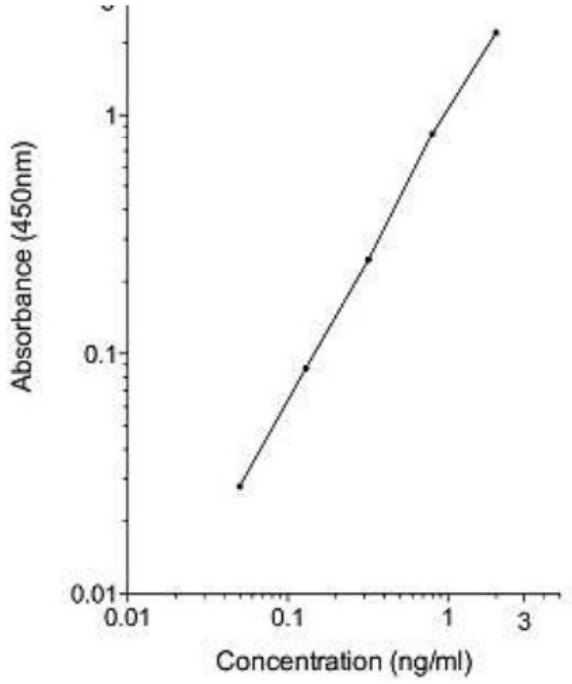
Calculation

1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against insulin concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Log-log curve-fitting is recommended for data analysis.
3. Determine insulin concentration of samples from standard curve.
4. Conversion factor: 1 µIU/ml = 0.0348 ng/ml.

Typical Standard Curve

Note: The following standard curve is provided for demonstration only. Do not use it to determine actual assay results. A standard curve should be generated for each assay.

Insulin(ng/ml)	Absorbance(450 nm)	Blanked Absorbance
0	0.054	0
3	0.085	0.031
7	0.14	0.086
16	0.32	0.266
40	0.939	0.885
100	2.521	2.467



Precision

Intra-assay Precision (Precision within an assay) C.V < 10%.

Inter-assay Precision (Precision between assays) C.V < 10%.

Detection Range

3 - 100 µIU/mL

Specificity

Percent of cross reactivity

Mouse insulin 100%

Rat insulin 100%