Equine Insulin Fast ELISA (Catalog# TBS34010)

DESCRIPTION

The Equine Insulin ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of equine insulin in serum, plasma, and cell culture supernatants. The microplate is coated with mouse monoclonal antibody specific for Insulin. Standards or samples are pipetted into the wells, and incubate together with biotinylated anti-insulin monoclonal antibody (Detection A). The insulin present in a sample is bound to the wells by the capture antibody, and then biotinylated antibody, producing an antibody-antigen-antibody "sandwich". After washing away unbound biotinylated antibody, HRP-streptavidin (Detection B) is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and exhibits a blue color in direct proportion to the amount of insulin in the initial sample. The reaction is terminated by the addition of an acid, and absorbance is measured at 450 nm. The concentration of insulin in the samples is determined by comparing the O.D. of the samples to the standard curve.

FEATURES:

(1) Rapid assay: Less than 3 hours.
(2) Every reagent is provided in liquid form and ready to use.
(3) Excellent precision and reproducibility.
(4) A simple assay procedure without any pretreatment of samples.

APPLICATIONS

Direct Assays: Insulin concentrations in different tissues.

KIT CONTENTS

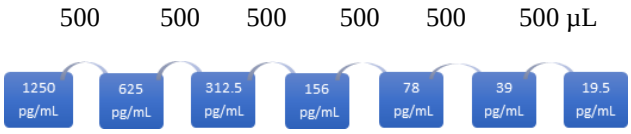
Reagents	Quantity
Precoated 96-well Plate	1
Standard	2x 100 µL
Detection A	1x 100 µL
Detection B	1x 100 µL
Sample Diluent (10 x concentrate)	1x 12 ml
Fast TMB Substrate	1x 12 ml
Stop Solution	1x 12 ml
Wash Solution (20 x concentrate)	1x 50 ml
Plate Sealers	4

Storage conditions: Store the kit at 4°C and standard at -20°C, respectively. Shelf life: 6 months after receipt.

PROCEDURES

Reagent Preparation:

1. Bring all reagents to room temperature (18-25˚C) before use.
2. Wash Buffer: Add 50 mL of 20x concentrated Wash Buffer into 950 mL distilled water and mix thoroughly.
3. Reagent Diluent: Dilute 10 mL of 10x concentrated Sample Diluent into 90 mL of deionized water (1:10) before use.
4. Dilute Detection B: Add 50 µL into 10mL of the Reagent Diluent for 96-well plate.
5. Standard: Set up 7 points of insulin standard concentrations: 1250, 625, 312.5, 156, 78, 39 and 19.5pg/mL as shown in the diagram below.
6. Label 7 tubes of 1.5ml from 1-7. Add 900 µL of the Reagent Diluents to the tube #1. Add 500 µL of the Reagent Diluents to tubes #2-7.
7. Add 100 µL of the insulin standard into tube #1 and vortex. This is Standard tube #1 with a concentration of 1250pg/mL.
8. Standards #2-7 are then prepared by performing a 1:2 dilution of the preceding standard. Mix each tube thoroughly before the next transfer. For example, to make Standard #2, remove 500 µL of Standard #1 and add it to tube #2 and vortex and so on. The Reagent Diluents serves as the zero standard (0 pg/ml).



Test Procedures

1. Add 100 µL of standards, blank and sample into plate in duplicate manner.
2. Add 5 µL of the Detection A to each well. Seal the plate and incubate with shaking for 2 hrs at room temperature.
3. Remove sealer and empty wells. Wash the plate 3 times with 150µL Wash Buffer.
4. Add 100µL of the diluted Detection B to each well. Seal the plate and incubate for 20 min at RT.

5. Repeat washing Step 3.

6. Add 100 µL of Substrate Solution to each well. Incubate for 20 min at RT. Protect from light.

7. Add 50 µL of Stop Solution to each well when the highest standard has developed a dark blue color. Gently tap the plate to ensure thoroughly mixing.

8. Determine the optical density of each well immediately, using a microplate reader set to 450nm.

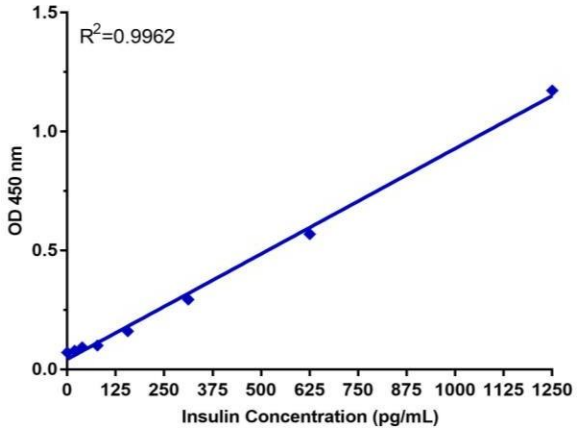
CALCULATION of Result

Average the duplicate readings for each standard, control and samples and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph. The data may be linearized by plotting the log of the insulin concentrations versus the log of the O.D. and the best-fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

- Mircorplate reader
- Pipetttes and Pipette tips
- Microtubes

Standard Curve of Equine Insulin



REPRODUCIBILITY

Intra-Assay CV%: <10%
Inter-Assay CV%: <12%

RELATED PRODUCTS:

BSA Standard solution (catalog# TBS5002)

Protein Cell Lysis Buffer (catalog# TBS5001)

Protein Assay Kit (Catalog# TBS2005)

TMB Substrate System (Catalog# TBS5021)