Rat Insulin ELISA Kit

Enzyme Immunoassay for the quantification of Insulin in serum and plasma

Catalog number: ARG80655

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INTRODUCTION

Insulin is one of the major regulatory hormones of immediate metabolism throughout the body. The biological actions of this hormone involve integration of carbohydrate, protein, and lipid metabolism. Insulin enhances membrane transport of glucose, amino acids, and certain ions. It also promotes glycogen storage formation of triglycerides and synthesis of proteins and nucleic acids. Immunocytochemical investigations have localized insulin in the B or β-cells of pancreatic islets of Langerhans. Deficiency of insulin results in diabetes mellitus, one of the leading causes of morbidity and mortality in the general population. Insulin is also present in tumors of β-cell origin such as insulinoma.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Streptavidin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells together with biotin-labeled highly specific monoclonal antibodies (capture antibodies). After 1 hour incubation with continuous agitation, the capture antibody-antigen complex is developed and immobilized to the pre-coated wells. After washing away any unbound substances, a HRP-conjugated monoclonal antibody specific to bind to another epitope of insulin is added to each well and incubate. After washing away any unbound reagents, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of inculin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 405nm and 450nm (620nm as reference

wavelength is recommended). The concentration of insulin in the sample is then determined by comparing the O.D of samples to the standard curve. The concentration of antigen is directly proportional to the optical density measured in the wells.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Streptavidin-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard	8 vials (S0-S7, ready-to-use) (0.156, 0.3125, 0.625, 1.25, 2.5, 5, 10 ng/ml)	4°C.
Biotin-labeled capture antibody	11 ml (Ready-to-use)	4°C
HRP-labeled conjugate detection antibody	13 ml (Ready-to-use)	4°C (Protect from light)
10X Wash buffer	50ml	4°C
TMB substrate	25 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	6 ml (Ready-to-use)	4°C
Cover sticks		

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and

assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (For eg., add 50ml wash buffer concentrate to 450ml distilled water). Upon dilution, the wash buffer should be stored at 2-8 °C where it is stable for 3 months.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 20 µl of standards and samples in duplicate into wells.
3. Add 100 µl of biotinylated capture antibody into each well. Cover with enclosed foil, incubate for 1h at RT with shaking at 300 rpm.
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting

against clean paper towels.

5. Add 125 µl of 1X HRP-labeled detection antibody solution to each well. Cover wells and incubate for 1h at RT with shaking at 300 rpm.
6. Aspirate each well and wash as step 4.
7. Add 200 µl of TMB Reagent to each well. Incubate for 30 minutes at room temperature in dark.
8. Add 50 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls or samples.

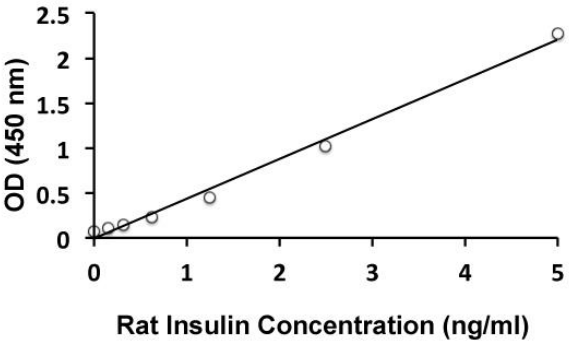
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

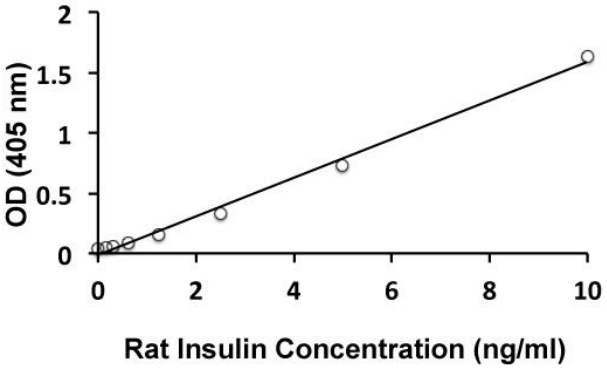
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



Typical Standard Curve at 450 nm



Typical Standard Curve at 405 nm

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of rat insulin ranged from 0.156 -10 ng/ml. The mean MDD was 0.093 ng/ml.

Specificity

Pig insulin: 31.0%

Bovine insulin: 76.0%

Human insulin: 46.0%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.9% and inter-assay precision was 6.7%.

Recovery

94-110%

PLATE LAYOUT