Rat Insulin ELISA Kit

Cat No. ELI-M-016-96

Size: 96 tests

MOLEQULE-ON®

Components	Quantity
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 Plate
Standard - lyophilized, 5.5ng/ml upon reconstitution	2 Vials
Detection antibody – 10ml/vial	1 Vial
Standard /sample Diluent - 10 ml/vial	1 Bottle
Wash Buffer Concentrate (20x) - 30 ml/vial	1 Bottle
Substrate Solution - 12 ml/vial	1 Bottle
Stop Solution - 12 ml/vial	1 Bottle
Plate Cover Seals	4 pieces

Reagent Preparations

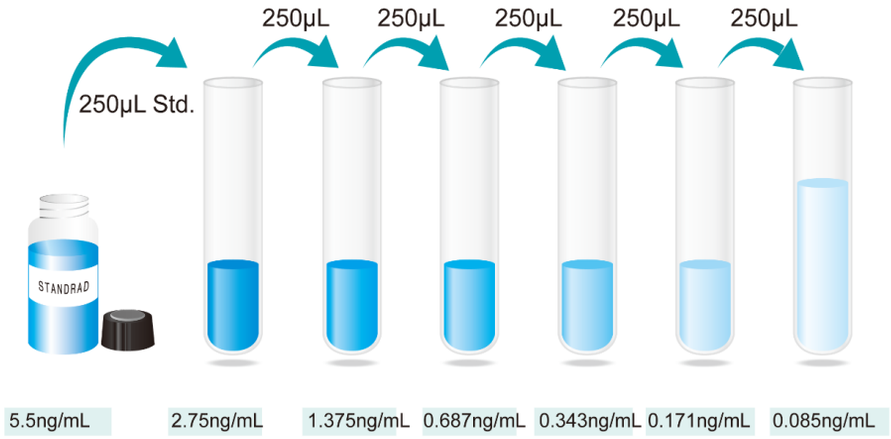
Temperature returning - Bring all kit components and specimen to room temperature (20-25℃) before use.

Wash Buffer - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Standard/Sample - Reconstitute the Standard with 0.5mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 5.5ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Standard/Sample Diluent into 2.75ng/ml tube and the remaining tubes. Use the stock solution of 5.5ng/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 5.5ng/mL standard serves as the high standard. The Standard/sample Diluent serves as the zero standard (0 pg/mL).

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

***The working solution should be used within one day after dilution.**



Storage

All components of Rat Insulin ELISA Kit should be store at 2-8°C except reconstituted standard that should be store at -20°C.

Description

Insulin is one of the most important hormones in glucose metabolism. The islet cells of the pancreas produce insulin precursor proteins, which are processed into c-peptides and insulin. They enter the blood circulation at equal molar concentrations. The mature insulin consists of two chains of a and b. These two chains are bridged by two disulfide bonds to form functional insulin molecules. The change of plasma glucose concentration is the most important stimulating factor for insulin production and secretion, and the insulin produced has some metabolic regulation. Its primary role is to transport sugar from peripheral blood to the liver for storage. Some factors, such as hepatic glycometogenesis disorders, or the effects of glucagon, epinephrine, growth hormone and cortisol, all antagonize the effect of insulin.

Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Insulin has been pre-coated onto a microplate. Standards, samples and horseradish peroxidase (HRP)-conjugated antibody are pipetted into the wells. Following extensive washing, tetramethyl-benzidine (TMB) reagent is added. Following a wash to remove any unbound combination, solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Sample Collection And Storage

Cell Culture Supernate - Centrifuge cell culture media at 1000 X g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000 X g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate 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.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

Procedure

1. Calculate and determine the number of strips needed for one experiment, the required strips are taken out and placed in the frame. Please put back the remaining strips into aluminum foil bag for seal and store them at 4 ℃.
2. It is suggested to set up a correction well, that is, the blank well. The method of setting the correction well is that only TMB reagent and stop solution are added to the well. In each experiment, the standard curve should be drawn at each time.
3. The sample or standard sample (10ul/well) of different concentration was added to the corresponding well, and the HRP-conjugate rat insulin antibody (100ul/well) was added quickly. The reaction well was sealed with the adhesive paper and incubated at room temperature (25~28℃) for 120 minutes.
4. Wash the plate 5 times. The last time you have to tap the micro-stripe on the clean absorbent paper, remove the residual washing fluid
5. 100ul TMB substrate solution was added to each well and incubated for 20 minutes at room temperature (25 ~ 28 ℃).
6. The enzyme reaction was terminated by adding 50ul stop solution to each well. OD value was measured at 450nm (reference wavelength 600-650nm) within 5 minutes.

Technical Note

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

Calculation of Results

1. The standard curve is used to determine the amount of sample.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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.
4. 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.

5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Standard (ng/ml)	OD.	OD.	Average	Corrected
0	0.098	0.096	0.097	-
0.085	0.153	0.156	0.155	0.058
0.171	0.277	0.265	0.271	0.174
0.343	0.401	0.394	0.398	0.301
0.687	0.534	0.552	0.543	0.446
1.375	0.792	0.775	0.784	0.687
2.75	1.449	1.472	1.461	1.364
5.5	2.33	2.365	2.348	2.251

Sensitivity, Specificity And Repeatability

SENSITIVITY: The minimum detectable dose was 0.1ng/mL.
SPECIFICITY: This assay recognizes both natural and recombinant rat Insulin. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.
REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY： The recovery of Insulin spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

References

1. Korner J, Savontaus E, Chua SC, Jr., Leibel RL, Wardlaw SL (2001) Leptin regulation of Agrp and Npy mRNA in the mousehypothalamus. J Neuroendocrinol 13:959-966
2. Olsson R and Carlsson PO (2005) Better vascular engraftment and function in pancreatic islets transplanted without prior culture. Diabetologia 48:469-476
3. Rydtren T and Sandler S (2002) Efficacy of 1400 W, a novel inhibitor of inducible nitric oxide synthase, in preventing interleukin-1beta-induced suppression of pancreatic islet function in vitro and multiple low-dose streptozotocin-induced diabetes in vivo. Eur J Endocrinol 147:543- 551 10