Rat Glucagon ELISA Kit

Cat No. ELI-M-017-96
Size: 96 tests

MOLEQULE-ON®

Components	Quantity
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 Plate
Standard - lyophilized, 2000pg/ml upon reconstitution	2 Vials
Concentrated Biotin-Conjugated antibody (100X) - 120 ul/vial	1 Vial
Concentrated Streptavidin-HRP solution (100X) - 120 ul/vial	1 Vial
Standard /sample Diluent - 16 ml/vial	1 Bottle
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 Bottle
Streptavidin-HRP Diluent - 16 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 200mL 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/Specimen**

Rat Glucagon Standard has a total of 2 vials. Each vial contains the standard sufficient for generating a standard curve. Reconstitute the Standard with 1.0mL of Standard/sample Diluent. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500uL of Standard/Sample Diluent into 1000pg/ml tube and the remaining tubes. Use the stock solution of 2000pg/mL to produce a 2-fold dilution series. Mix each tube thoroughly (vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 2000 pg/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.**

- **Working solution of Biotin-Conjugate anti-Rat Glucagon antibody**

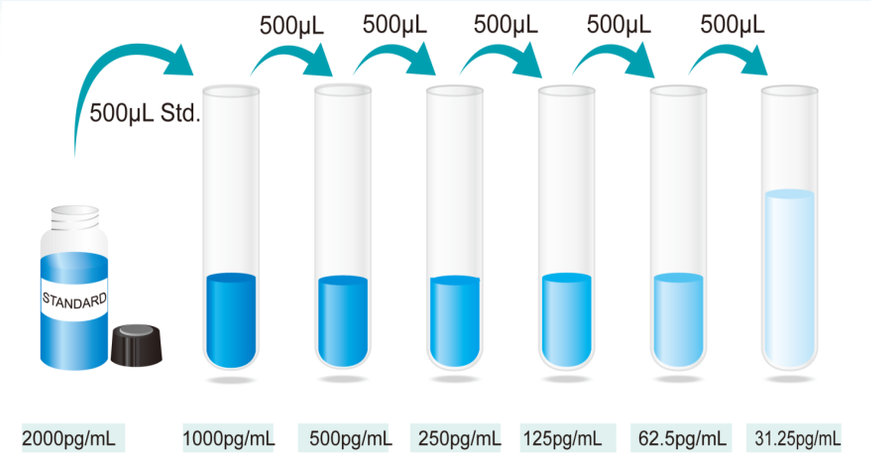
The lyophilized Detection Antibody should be stored at 4℃ to -20℃ in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 110 µL of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take 100µL of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used, make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

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

- **Working solution of Streptavidin-HRP (120µL)**

Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120 µL HRP Conjugate sufficient for a 96-well plate. Make a 1:100 dilution in Reagent Diluent. If the entire 96-well plate is used, add 100µL of HRP Conjugate to 10 mL of Streptavidin-HRP Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4° C for up to 6 months. DO NOT FREEZE.

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



Storage

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

Description

Glucagon is a peptide hormone, produced by alpha cells of the pancreas. It works to raise the concentration of glucose in the bloodstream. Its effect is opposite that of insulin, which lowers the glucose. The pancreas releases glucagon when the concentration of glucose in the bloodstream falls too low. Glucagon causes the liver to convert stored glycogen into glucose, which is released into the bloodstream. High blood-glucose levels stimulate the release of insulin. Insulin allows glucose to be taken up and used by insulin-dependent tissues. Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level. It increases energy expenditure and is elevated under conditions of stress. Glucagon belongs to a family of several other related hormones. Glucose is stored in the liver in the form of the polysaccharide glycogen, which is a glucan (a polymer made up of glucose molecules). Liver cells (hepatocytes) have glucagon receptors. When glucagon binds to the glucagon receptors, the liver cells convert the glycogen into individual glucose molecules and release them into the bloodstream, in a process known as glycogenolysis. As these stores become depleted, glucagon then encourages the liver and kidney to synthesize additional glucose by gluconeogenesis. Glucagon turns off glycolysis in the liver, causing glycolytic intermediates to be shuttled to gluconeogenesis. Glucagon also regulates the rate of glucose production through lipolysis. Glucagon induces lipolysis in rats under conditions of insulin suppression (such as diabetes mellitus type 1). Glucagon binds to the glucagon receptor, a G protein-coupled receptor, located in the plasma membrane. The conformation change in the receptor activates G proteins, a heterotrimeric protein with α, β, and γ subunits.

Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Glucagon has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Glucagon present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for Glucagon is added to detect the captured Glucagon protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. 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×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°C. Centrifuge at approximately for 15 minutes at 1000×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×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. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.
3. Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350µl) using a multichannel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100µl working solution of Biotin-Conjugate anti- Rat Glucagon antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature (25±2℃).
6. Repeat step 4.
7. Add 100µl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 20 minutes at room temperature(25±2℃).
8. Repeat step 4 with an additional wash.
9. Add 100µl Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature (25±2℃). Protect from light.
10. Add 50µl Stop solution to each well. Gently tap the plate to ensure thorough mixing.
11. Read at 450nm 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 specimens.
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 Glucagon 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.

Std (pg/mL)	O.D.1	O.D.2	Average	Corrected
0	0.072	0.073	0.073	---
31.25	0.117	0.119	0.118	0.045
62.5	0.160	0.164	0.162	0.089
125	0.247	0.251	0.249	0.176
250	0.417	0.424	0.421	0.348
200	0.769	0.773	0.771	0.698
1000	1.383	1.388	1.386	1.313
2000	2.480	2.484	2.482	2.409

Sensitivity, Specificity And Repeatability

SENSITIVITY: The minimum detectable dose was 6 pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant Rat Glucagon.
REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY： The recovery of Glucagon spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

References

1. White CM (1999). Journal of Clinical Pharmacology 39 (5): 442–7.
2. Jones, BJ, et al. (2012). Endocrinology 153 (3): 1049–54. doi:10.1210/en.2011-1979. 3.
3. Liljenquist JE, et al. (1974). The Journal of Clinical Investigation 53 (1): 190–7.