Rat Insulin ELISA Kit

Cat. No.:DEIA1172
Pkg.Size:96T

Intended use

The Rat Ins ELISA kit is designed to detect and quantify the level of Rat Ins in cell culture supernatant, serum, plasma and tissue. This kit is for research use only and not intended for diagnostic purposes.

General Description

Insulin is one of the major regulatory hormones of intermediate 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 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 B cell origin such as insulinoma.

Principle Of The Test

A monoclonal antibody specific for Rat Ins has been coated onto the wells of the microtiter strips provided. Standards and samples are pipetted into the wells and Rat Ins binds to the immobilized antibody. After incubation, unbound substances is removed during a wash step, a biotin-conjugated anti-Rat Ins antibody is added and binds to Rat Ins captured by the first antibody. After incubation, unbound biotin-conjugated anti-Rat Ins antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-Rat Ins antibody. After incubation, unbound streptavidin-HRP is removed during a wash step, and a chromogenic substrate solution is added to the wells and color develops in proportion to the amount of Ins bound in the initial step. A colored product is formed and the reaction is terminated by the addition of stop solution. The intensity of the color is measured spectrophotometrically at 450 nm.

Materials Required But Not Supplied

1. A standard ELISA plate reader for absorbance at 450 nm.
2. Calibrated adjustable precision pipettes (single channel and multi channel), preferably with disposable plastic tips.
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software or graph paper.
6. Polypropylene tubes.
7. Graduated cylinders and calibrated beakers in various sizes.

Storage

Store all reagents at 2 to 8℃.

Specimen Collection And Handling

1. The serum/plasma must be separated from the clot as early as possible as to avoid hemolysis. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters.

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2. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.
3. Avoid repeated freeze-thaw cycles.
4.It is recommended that all samples be diluted in Assay Solution, and the exact dilution must be determined empirically.

Precautions

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.
1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. CAUTION - CRITICAL STEP: Allow the reagents and samples to stabilize at room temperature (18-30 ℃) before use. Shake reagent gently before, and return to 2-8 ℃ immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air -bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37℃ inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to rabies virus. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121℃ or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M H_2SO_4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. Proclin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

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