Insulin (Canine) ELISA Kit

Catalog Number KA2291

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The canine Insulin ELISA test is an immunoassay designed for the quantitative determination of circulating Insulin, a peptide hormone in serum/plasma samples of canine and related species. The test is intended for professional use as an aid in the diagnosis and monitoring of physiological/pathological conditions related to circulating Insulin.

Background

Insulin is a peptide hormone very intimately involved in the control and regulation of all cellular activities of carbohydrate homeostasis, and is secreted by the beta cells of the pancreas. The circulating sugars intern insert a feedback regulation on the secretion of Insulin. Physiological circulating levels of Insulin is very important for many cellular, organ and body functions. Any changes in levels leads to diabetes and related complex cycle of pathological events.

Principle of the Assay

The Insulin ELISA Test Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes anti-Insulin antibodies for solid phase (microtiter wells) immobilization and a mouse monoclonal antibodies in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in Insulin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minute incubation period, at 37°C, the wells are washed with wash buffer to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the absorbency is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is directly related to the amount of unlabeled Insulin in the sample. By reference to a series of Insulin standards assayed in the same way, the concentration of Insulin in the unknown sample is computed and quantified.

General Information

Materials Supplied

List of component

Component	Amount
Antibody-coated microtiter wells	96 wells/plate
Reference Standard (0, 1, 2.5, 5, 10, 25 ng/mL) (Lyophilized): Reconstitute in 1 mL Standard diluent.	6 vials
Enzyme Conjugate Reagent (Lyophilized HRP)	1 vial
HRP Diluent (Enzyme-Conjugate Diluent)	12 mL
Standard/Sample diluent	20 mL
TMB Color Reagent (ready to use)	12 mL
Stop solution (2N HCl)	6 mL
20x Wash buffer	20 mL

Storage Instruction

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10 nm or less, with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at a 450 nm wavelength is acceptable for use in absorbency measurement.

Materials Required but Not Supplied

- ✓ Precision pipettes: 50 uL, 100 uL, 200 uL, and 1.0 mL
- ✓ Disposable pipette tips
- ✓ Distilled water
- ✓ Glass tubes or flasks to prepare TMB Solution
- ✓ Vortex mixer or equivalent
- ✓ Absorbent paper of paper towel
- ✓ Graph paper
- ✓ Microtiter plate reader

Precautions for Use

- ✓ For Research Use Only.

Assay Protocol

Reagent Preparation

- ✓ All reagents should be brought to room temperature (18-25°C) before use.
- ✓ To prepare the wash buffer add one part of the reagent buffer to 19 parts of distilled water. Prepare desired amount and excess solution can be stored (refrigerated) and is stable for one week.
- ✓ Lyophilized standards should be reconstituted in 1 mL and stored, sealed, at 2-8°C. If not used for long term, should be stored at -20°C.
- ✓ The HRP 1 vial provided as Lyophilized should be reconstituted in 12 mL or desired volume can be stored at -20°C for long term use, avoid frequent thaw/freeze cycles.

Sample Preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

Assay Procedure

One must follow accurately these steps to ensure correct results. Use clean pipettes and sterile, disposable tips:

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µL of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µL of Enzyme Conjugate into each well. Mix for 30 seconds. It is very important to have complete mixing at this step, use the shaker.
4. Incubate at 37°C incubator for 1 hour.
5. Remove the incubation mixture by dumping plate contents into a waste container.
6. Rinse and dump the microtiter wells five (5) times with wash buffer.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100 µL of TMB solution into each well. Gently mix for 10 seconds.
9. Incubate at room temperature for 20 minutes, in the dark.
10. Stop reaction by adding 50 µL of 2N HCl to each well.
11. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
12. Read optical density at 450 nm with a microtiter well reader.

Data Analysis

Calculation of Results

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and samples. Construct a standard curve by plotting the mean absorbency obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbency values on the vertical or Y axis, and concentrations on the horizontal or X axis. Use the mean absorbency values for each specimen to determine the corresponding concentration of Insulin in ng/ml from the standard curve.

- Limitation of the test

✓ Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

✓ The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

✓ The results obtained from the use of this kit should be used for research only.

✓ Avoid using highly hemolytic and lipofilic samples. Also, avoid freeze and thaw samples repeatedly, as the peptides hormones are denatured and the analysis will give you false results.

Performance Characteristics

- Sensitivity and expected values

Each laboratory must establish its own normal ranges based on your in-house data The minimal detectable concentration of canine Insulin by this assay is estimated to be 0.5 ng/mL.

Resources

References

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Plate Layout

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