Canine GROWTH HORMONE

Canine GH ELISA TEST KIT

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE

Canine GH ELISA is intended to quantitative determination of Growth Hormone (cGH) concentration in serum/plasma of Canine and related species. The test is designed as research tool in evaluation of preclinical samples in Canine and related species and should be employed by a trained/skilled professional.

INTRODUCTION

Growth Hormone (is also called somatotropin) is secreted by the anterior pituitary gland and is under the influence of hypothalamic Growth Hormone Releasing Factor (GHRF). It has 191 amino acids in length and has a molecular mass of approximately 22,000 daltons. There are a few structural similarities of growth hormone between species. Its metabolic effects are primarily anabolic. For example, human GH promotes protein conservation and is engaged in a wide range of mechanisms for protein synthesis. It also enhances glucose transport and facilitates glycogen storage. Another family of peptide hormones, the somatomedins, mediates its cascade of growth-promoting action. Many factors are known to influence the rate of growth hormone secretion, including periods of sleep and wakefulness, exercise, stress, hypoglycemia, estrogens, corticosteroids and L-dopa.

The Growth hormones and Prolactins vary slightly in structure among various species

The Canine Growth Hormone Enzyme Immunoassay provides rapid, sensitive and reliable results.

TEST PRINCIPLE

The cGH Quantitative Test Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a mouse anti-cGH specific antibody for solid phase (microtiter wells) immobilization and high affinity antimouse - CGH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in cGH molecules being sandwiched between the solid phase and enzyme -linked antibodies. After 3 hours of incubation at 37C, the wells are washed with water 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 stop solution, and the color is changed to yellow and measured spectrophotometrically at 450nm. The concentration of cGH is directly proportional to the color intensity of the test sample.

MATERIALS PROVIDED

1. Antibody-coated microtiter wells, 96-well plate
2. Enzyme -Conjugate reagent 12 mL
3. Reference Standards (0, 1.0, 2.5, 5, 10, 25, 50 ng/mL)
4. TMB Color Reagent, 12 mL
5. Stop solution (2N HCl), 6 mL
6. 20x Washing Buffer, 20mL.
7. Instructions

MATERIALS REQUIRED, BUT NOT PROVIDED

1. Precision pipettes: 50uL, 100uL, 200uL, and 1.0mL
2. Disposable pipette tips
3. Distilled water
4. Glass tubes or flasks to prepare TMB Solution
5. Vortex mixer or equivalent
6. Absorbent paper
7. Graph paper
8. Microtiter plate reader

SPECIMEN COLLECTION AND PREPARATION

Serum/plasma should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum or plasma samples only and not for whole blood. The Canine test samples (plasma or serum) should be collected fresh and repeated frozen and thawed samples should be avoided. If the test samples are not analyzed immediately, should be stored at -20C in small aliquots and take one aliquot at a time for analysis.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 4-8^{O}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 10nm or less, with a bandwidth of 10nm or less and an optical density range of 0-3.0 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25^{O}C) before use.
2. Ready to use reference standards is provided with the kit. The standards are stable at 2-8^{O}C for 2 weeks or kept frozen at -20C for long term use.

ASSAY PROCEDURE

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

1. Secure desired number of coated wells in the holder.
2. Dispense 100 ul of standards, specimens, and controls into appropriate wells.
3. Dispense 100ul of Enzyme Conjugate Reagent into each well. Shake the plate for 30 seconds. It is very important to shake the plate very well at this step.
4. Incubate at room temperature (18-25^{O}C) for 3 hours.
5. Remove the incubation mixture by dumping plate contents into a waste container.
6. Rinse and dump the microtiter wells five (5) times (200-300ul) with dilute wash buffer.
7. Dispense 100 ul of TMB solution into each well. Gently mix for 10 seconds.
8. Incubate at room temperature for 20 minutes in the dark.
9. Stop reaction by adding 50 ul of stop solution (2N HCl) to each well.

10. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
11. Read optical density at 450nm with a microtiter well reader.

Important note: The wash steps are very critical and insufficient washing will result in poor precision and falsely elevated absorbency readings.

CALCULATION OF RESULTS

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbency obtained from each reference standard against its concentration in ng/ml on graph , 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 cGHng/mL from the standard curve.

EXPECTED VALUES AND SENSITIVITY

It is recommended to establish your local laboratory conditions for normal range in your laboratory animals.
Minimum detectable levels in this assay will be 0.25ng/mL

LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained, when the assay procedures are carried out with understanding of the package insert instructions and adherence to good laboratory practice.
The wash step is extremely important and should be followed for clean background and good reproducible results. Incubation conditions should be carefully monitored or establishing conditions at 37C should make adjustments for consistent and reproducible results. Note: The components of this kit should not be mixed are used with other manufacturer kits.

Limitations & Warranty

The present ELISA is designed for helping the scientist to analyze test samples only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Endocrine Technologies, Inc. is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. Warranty is limited to replacement of similar ELISA Kit damaged during shipment or leaking solutions within 30 days, with written explanation and return of the ELISA product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.

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A TYPICAL DOSE RESPONSE CURVE FOR K9 GH

	OD@450
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.1291
TOP	9.775
LOGEC50	2.407
HILLSLOPE	0.9672
EC50	255.3
Std. Error	
BOTTOM	0.02962
TOP	4.344
LOGEC50	0.3214
HILLSLOPE	0.1062
95% Confidence Intervals	
BOTTOM	0.04684 to 0.2113
TOP	-2.284 to 21.83
LOGEC50	1.515 to 3.299

GROWTH HARMONE STANDARD CURVE