GH ELISA Kit

Catalog Number KA2279

96 assays

Version: 09

Intended for research use only

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Introduction

Intended Use

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

Background

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 GH (Bovine) ELISA Kit provides rapid, sensitive, and reliable results.

Principle of the Assay

The GH (Bovine) ELISA Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes anti-bGH specific antibody for solid phase (microtiter wells) immobilization and anti-bGH antibody enzyme (horseradish peroxidase) conjugate for detection system. The test sample is allowed to react simultaneously with the antibodies, resulting in bGH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 3 hours of incubation at 37°C, 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 450 nm. The concentration of bGH is directly proportional to the color intensity of the test sample.

General Information

Materials Supplied

List of component

Component	Amount
Antibody-coated micro titer wells	96 wells
HRP-Enzyme Conjugate	12 mL
Lyophilized Standards (0, 1.0, 2.5, 10, 25, 50 ng/mL), reconstitute in 1 mL standard/sample diluent.	1 set
Standard/Sample Diluent	20 mL
TMB Color Reagent	12 mL
Stop solution (2 N HCl)	6 mL
20X Wash buffer	20 mL

Storage Instruction

Unopened test kits should be stored at 4-8°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.

Materials Required but Not Supplied

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

Precautions for Use

This product is for Research Use Only.

Please read the entire protocol carefully before starting your experiment.

✓ Limitation 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 established conditions at 37°C should make adjustments for consistent and reproducible results.
- The components of this kit should not be mixed are used with other manufacturer kits.

✓ Limitations and Warranty

The present ELISA is designed for helping the scientist to analyze test samples from bovine species only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Abnova is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.

Assay Protocol

Reagent Preparation

- ✓ All reagents should be brought to room temperature (18-25°C) before use.
- ✓ Lyophilized standards should be diluted in 1 mL Standard/Sample diluent. This can be stored at -20°C for long term use.

Sample Preparation

Serum/plasma should be prepared from a whole blood specimen obtained by acceptable techniques. This kit is for use with serum or plasma samples only and not for whole blood. The bovine 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 -20°C in small aliquots and take one aliquot at a time for analysis.

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 200 µL of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µL 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°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 (200-300 µL) with dilute wash buffer.
7. Dispense 100 µL 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 µL of stop solution (2 N 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 450 nm 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.*

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, 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 bGH ng/mL from the standard curve.

Performance Characteristics

✓ 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 0.2 ng/mL.

Resources

References

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3. Goldfine ID 1978 Medical treatment of acromegaly Ann Rev of Med. 29, p407
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