Fish Growth Hormone(GH) ELISA Kit

Catalog No. CSB-E12121Fh

(96 tests)

- This immunoassay kit allows for the in vitro rapid detection of Fish GH concentrations in **serum, plasma** and other biological fluids.
- **Expiration date** six months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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INTRODUCTION

Growth hormone is a protein hormone of about 190 amino acids that is synthesized and secreted by cells called somatotrophs in the anterior pituitary. It is a major participant in control of several complex physiologic processes, including growth and metabolism. Growth hormone is also of considerable interest as a drug used in both humans and animals.

Growth is a very complex process, and requires the coordinated action of several hormones. The major role of growth hormone in stimulating body growth is to stimulate the liver and other tissues to secrete IGF-I. IGF-I stimulates proliferation of chondrocytes (cartilage cells), resulting in bone growth. Growth hormone does seem to have a direct effect on bone growth in stimulating differentiation of chondrocytes. IGF-I also appears to be the key player in muscle growth. It stimulates both the differentiation and proliferation of myoblasts. It also stimulates amino acid uptake and protein synthesis in muscle and other tissues.

Growth hormone has important effects on protein, lipid and

carbohydrate metabolism. In some cases, a direct effect of growth hormone has been clearly demonstrated, in others, IGF-I is thought to be the critical mediator, and some cases it appears that both direct and indirect effects are at play.

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. A antibody specific to GH has been pre-coated onto a microplate. Standards or samples are added to the appropriate microtiter plate wells with biotin-conjugated GH and incubated. A competitive inhibition reaction is launched between GH (Standards or samples) and Biotin-conjugated GH with the pre-coated antibody specific for GH. The more amount of GH in samples, the less antibody bound by Biotin-conjugated GH. Then Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of GH in the sample. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

The standard curve concentrations used for the ELISA's were 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312pg/ml.

SPECIFICITY

This assay recognizes GH. No significant cross-reactivity or interference was observed.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Standards (S1-S5)	5
HRP-avidin	1 x 6 ml
Conjugate	1 x 6 ml
Wash Buffer	1 x 15 ml (20×concentrate)
Substrate A	1 x 6 ml
Substrate B	1 x 6 ml
Stop Solution	1 x 6 ml

Standard	S1	S2	S3	S4	S5
Concentration(pg/ml)	312	625	1250	2500	5000

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

TECHNICAL HINTS

1. Centrifuge vials before opening to collect contents.
2. Bring all reagents and plate to room temperature for at least 30 minutes before use. Unused wells need store at 2-8℃and avoid sunlight.

3. **Wash Buffer** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 300 ml of Wash Buffer.
4. 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.
5. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
6. Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
7. Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.
- **Plasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.

1. Set a Blank well without any solution. Add 50μl of Standard or Sample per well. Standard need test in duplicate.
2. Add 50μl of **Biotin-conjugate** GH to each well (not to Blank well), Mix well and then incubate for 1 hour at 37°C.
3. Fill each well with Wash Buffer (about 200μl), stay for 10 seconds and Spinning. Repeat the process for a total of three washes. 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.
4. Add 50μl of **HRP-avidin** to each well. Incubate for 30mins at 37°C.
5. Repeat the aspiration and wash five times as step 4.
6. Add 50μl of **Substrate A** and **Substrate B** to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
7. Add 50μl of **Stop Solution** to each well.
8. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, Blank, and sample and subtract the optical density of Blank. Create 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. The data may be linearized by plotting the log of the GH 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.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.

- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in Standard Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.