Fish GH Immunoassay

Catalog Number: SEKFM-0001

For the quantitative determination of Fish GH concentrations in cellculture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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TABLE OF CONTENTS

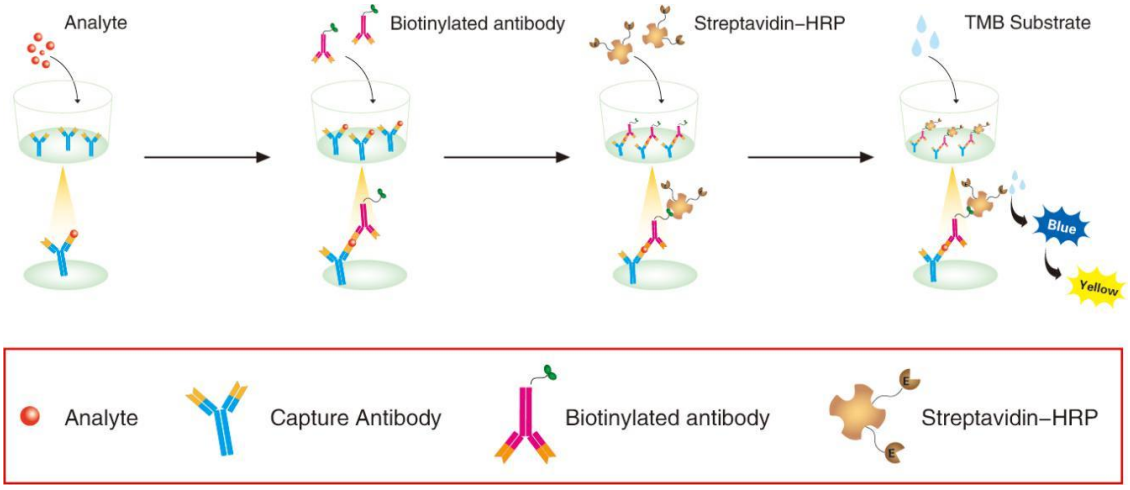
BACKGROUND

Fish GH is a single chain protein hormone secreted by eosinophils in the anterior lobe of the pituitary gland, which consists of 173 to 188 amino acids and has a molecular weight of 20000 to 22000 Daltons. Fish GH has some homology with other vertebrates GH in terms of molecular weight, amino acid composition and sequence. GH is a key regulatory factor in the growth and development of fish body. GH can act on almost all tissues of the body, stimulate tissue development and increase the size and number of somatic cells. In addition, also has the regulation of fish metabolism, enhance immunity, promote cell division.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific forGHhas been pre-coated onto a microplate. Standards and samples are pipetted into the wells and anyGHpresent is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for GHis added to detect the captured GHprotein in sample. For signal development, horseradish peroxidase (HRP)-conjugatedStreptavidinis added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



TECHNICAL HINTSAND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit

label.

2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate-antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,50ng/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
ConcentratedBiotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
ConcentratedStreptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard /Sample Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent- 16ml/vial	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.

2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates- Centrifuge cell culture media at 1000×gto remove debris. Assay immediately oraliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

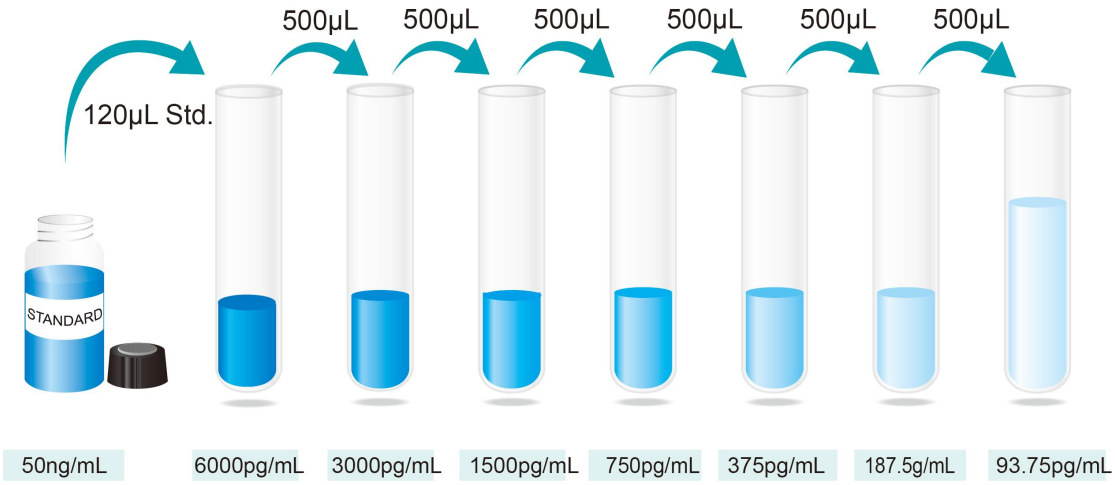
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at roomtemperature or overnight at 2-8℃. Centrifugeapproximately for 15 minutes at 1000×g. Assayimmediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and storesamples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: The normal Fish serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentratewith 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mixgently until the crystals have completely dissolved.
3. **Standard/Specimen**- Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 50ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 120μL ofStandard/Sample Diluent into6000pg/ml tube(880ul) and the remaining tubes. Use the stock solution of 6000pg/mL to produce a 2-folddilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 6000pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of GH standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-Fish GH antibody:**Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃)..

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-Fish GH antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 30 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 10-20 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

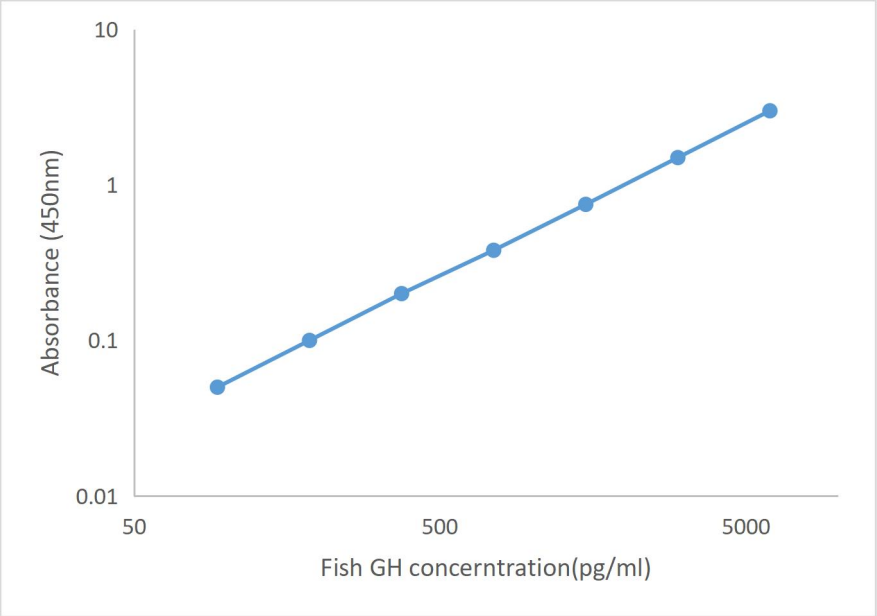
1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using 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.
4. The data may be linearized by plotting the log of the GHconcentrations 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.

5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the GH ELISA

Standard(pg/ml)	OD.	OD.	Average	Corrected
0.00	**0.052**	**0.056**	**0.054**	**---**
93.75	**0.096**	**0.103**	**0.099**	**0.045**
187.5	**0.189**	**0.193**	**0.191**	**0.137**
375	**0.312**	**0.351**	**0.331**	**0.277**
750	**0.496**	**0.531**	**0.513**	**0.459**
1500	**0.758**	**0.734**	**0.746**	**0.692**
3000	**1.389**	**1.345**	**1.367**	**1.313**
6000	**2.429**	**2.461**	**2.445**	**2.391**



Representative standard curve for GH ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 46pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant Fish GH.

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of GH spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of GHin two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	89	81-105
Cell culture supernatants	96	90-112

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of GH in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	92	102
	Range (%)	80-103	96-113
1:4	Average% of Expected	91	104
	Range (%)	90-112	101-114
1:8	Average% of Expected	94	105
	Range (%)	83-104	96-111
1:16	Average% of Expected	93	104
	Range (%)	88-101	94-113

REFERENCES