Fish Follicle-stimulating hormone (FSH) ELISA Kit

Catalog Number: EK10442

For the quantitative detection of Fish Follicle-stimulating hormone (FSH) concentration in serum, plasma and other biological fluids.

This instruction must be read in its entirety before using this product.

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

Contact information

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REAGENTS AND MATERIALS PROVIDED

Reagents	Quantity	Reagents	Quantity
Assay plate (96 Wells)	1	Instruction manual	1
Standards	6 x 1 mL	Antibody	1 x 6 mL
HRP-Conjugate	1 x 6 mL	Wash Buffer (20 x concentrate)	1 x 15 mL
Substrate A	1 x 7 mL	Substrate B	1 x 7 mL
Stop Solution	1 x 7 mL	Adhesive Films	4

MATERIALS REQUIRED BUT NOT SUPPLIED

※ Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm.

※ Precision single or multi-channel pipettes and disposable tips.

※ Deionized or distilled water.

※ Eppendorf Tubes for serial dilution samples.

※ Container for Wash Solution.

※ Absorbent paper for blotting the microtiter plate.

STORAGE

<table>
<tr><td>Unopened kit</td><td colspan="2">Store at 2 - 8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Coated assay plate</td><td>May be stored for up to 1 month at 2 - 8°C. Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal, and avoid the damp.</td></tr>
<tr><td>Standard</td><td rowspan="7">May be stored for up to 1 month at 2 - 8°C.</td></tr>
<tr><td>Antibody</td></tr>
<tr><td>HRP-Conjugate</td></tr>
<tr><td>Wash Buffer</td></tr>
<tr><td>Substrate A</td></tr>
<tr><td>Substrate B</td></tr>
<tr><td>Stop Solution</td></tr>
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INTRODUCTION

Follicle-stimulating hormone (FSH) is a hormone found in humans and other animals. It is synthesized and secreted by gonadotrophs of the anterior pituitary gland. FSH regulates the development, growth, pubertal maturation and reproductive processes of the body. FSH and luteinizing hormone (LH) act synergistically in reproduction.

FSH is a 35.5 kDa glycoprotein heterodimer, consisting of two polypeptide units, alpha and beta. Its structure is similar to those of luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG). The alpha subunits of the glycoproteins LH, FSH, TSH, and hCG are virtually identical and consist of about 96 amino acids, while the beta subunits vary. Both subunits are required for biological activity. FSH has a beta subunit of 111 amino acids (FSH β), which confers its specific biologic action, and is responsible for interaction with the follicle-stimulating hormone receptor. The sugar portion of the hormone is covalently bonded to asparagine, and is composed of N-acetylgalactosamine, mannose, N-acetylglucosamine, galactose, and sialic acid, the last one being critical for its biological half-life of 3-4 hours.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with goat-anti-rabbit antibody. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for FSH and Horseradish Peroxidase (HRP) conjugated FSH. The competitive inhibition reaction is launched between with HRP labeled FSH and unlabeled FSH with the antibody. A substrate

solution is added to the wells and the color develops in opposite to the amount of FSH in the sample. The color development is stopped and the intensity of the color is measured

LIMITATIONS OF THE PROCEDURE

- ※ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- ※ The kit should not be used beyond the expiration date on the kit label.
- ※ Do not mix or substitute reagents from different sources or lots.
- ※ 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 ELISA Kit, the possibility of interference cannot be excluded.

DETECTION RANGE

2 mIU/mL - 140 mIU/mL. The standard curve concentrations used for the ELISA's were 140 mIU/mL, 60 mIU/mL, 20 mIU/mL, 6 mIU/mL, 2 mIU/mL, 0 mIU/mL.

SENSITIVITY

The limit of detection of Fish FSH defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 1 mIU/mL (mean of 6 independent assays).

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of Fish FSH. No significant cross-reactivity or interference between Fish FSH and analogues was observed.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between Fish FSH and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

CV(%) = SD/meanX100

Intra-Assay: CV<8%

Inter-Assay: CV<12%

STABILITY

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage condition.

The loss rate was determined by accelerated thermal degradation test. Keep the kit at 37°C for 4 and 7 days, and compare O.D.values of the kit kept at 37°C with that of at recommended temperature. (referring from China Biological Products Standard, which was calculated by the Arrhenius equation. For ELISA kit, 4 days storage at 37°C can be considered as 6 months at 2-8°C, which means 7 days at 37°C equaling 12 months at 2-8°C).

Note:

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 2-8°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at ⩽ -20°C. Avoid repeated freeze-thaw cycles.
- **Plasma** Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 ×g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ⩽ -20°C. Avoid repeated freeze-thaw cycles.
- **Other biological fluids** Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen can not be detected.
3. When performing the assay, bring samples to room temperature.

SAMPLE PREPARATION

Fish serum or plasma samples require no dilution before test. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer (1 x) - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 mL of Wash Buffer Concentrate (20 x) into deionized or distilled water to prepare 300 mL of Wash Buffer (1 x).

Tube	S5	S4	S3	S2	S1	S0
mIU/mL	140	60	20	6	2	0

ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal, store unused wells at 2 - 8°C.
3. Set a Blank well without any solution. Add 50 µL of Standard or Sample to per well. Add 50 µL of HRP-Conjugate to each well (Note: Do not add to Blank!), then 50 µL Antibody to each well. Mix well, Cover with the adhesive films provided. Incubate for 1 hour at 37°C.
4. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. 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.

5. Add 50 µL of Substrate A and 50 µL of 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. Avoid placing the plate in direct light.
6. Add 50 µL of Stop Solution to each well. when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm. Subtract readings at 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

***Samples may require dilution. See Sample Preparation section.**

CALCULATION OF RESULTS

Using the professional soft "Curve Exert 1.4" to make a standard curve is recommended.

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.
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 x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the FSH 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.

IMPORTANT NOTE

1. The instruction manual also suits for the kit of 48T, but all reagents of 48T kit are reduced by half.
2. There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.
3. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
4. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. 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. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
6. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. 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.
7. Controlling of reaction time: Observe the change of color after adding Substrate Solution (e.g. observation once every 10 minutes), Substrate Solution should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

8. Substrate Solution is easily contaminated. Substrate Solution should remain colorless or light blue until added to the plate. Please protect it from light.
9. 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.
10. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.
11. Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement.
12. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.

13. Limited by the current condition and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
14. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
15. Kits from different batches may be a little different in detection range, sensitivity and color developing time.
16. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
17. Kits from different manufacturers with the same item might produce different results, since we haven’t compared our products with other manufacturers.
18. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
19. Valid period: six months.