Human FSH ELISA Kit

Enzyme Immunoassay for the quantitative determination of human Follicle Stimulating Hormone (FSH) in serum

Catalog number: ARG80844

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are intimately involved in the control of the growth and reproductive activities of the gonadal tissues, which synthesize and secrete male and female sex hormones through a negative feedback relationship.

FSH is a glycoprotein secreted by the basophil cells of the anterior pituitary. Gonadotropin-releasing hormone (GnRH), produced in the hypothalamus, controls the release of FSH from the anterior pituitary. Like other glycoproteins, such as LH, TSH, and HCG, FSH consists of subunits designated as alpha and beta. Hormones of this type have alpha subunits that are very similar structurally, therefore the biological and immunological properties of each are dependent on the unique beta subunit. In the female, FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells; follicular steroidogenesis is promoted and LH production is stimulated. The LH produced then binds to the theca cells and stimulates steroidogenesis. Increased intraovarian estradiol production occurs as follicular maturation advances, thereupon stimulating increased FSH receptor activity and FSH follicular binding. FSH, LH, and estradiol are therefore intimately related in supporting ovarian recruitment and maturation in women.

FSH levels are elevated after menopause, castration, and in premature ovarian failure. The levels of FSH may be normalized through the administration of estrogens, which demonstrate a negative feedback mechanism. Abnormal relationships between FSH and LH, between FSH and estrogen have been linked to anorexia nervosa and polycystic ovarian disease. Although there are significant exceptions ovarian failure is indicated when random FSH

concentrations exceed 40 mIU/mL. The growth of the seminiferous tubules and maintenance of spermatogenesis in men are regulated by FSH. However, androgens, unlike estrogens, do not lower FSH levels, therefore demonstrating a feedback relationship only with serum LH. For reasons not fully understood, azospermic and oligospermic males usually have elevated FSH levels. Tumors of the testes generally depress serum FSH concentrations, but levels of LH are elevated, as determined by radioimmunoassay. It has been postulated that the apparent LH increase may be caused by crossreactivity with hCG-like substances secreted by tumors of the testes. High levels of FSH in men may be found in primary testicular failure and Klinefelter syndrome. Elevated concentrations are also present in cases of starvation, renal failure, hyperthyroidism, and cirrhosis.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for FSH has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any FSH present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for FSH is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of FSH bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of FSH in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-FSH antibody Conjugate	11 ml (ready to use)	4°C
Standards 0-5 (0, 5, 10, 20, 50, 100 mIU/ml)	6 vials (lyophilized)	4°C
TMB substrate	14 ml (ready to use)	4°C (Protect from light)
STOP solution	14 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- All kit reagents and specimens should be brought to room temperature (21-26°C) and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and

devices are prepared ready at the appropriate time.

- Ensure complete reconstitution and dilution of reagents prior to use.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Store the unopened reagents at 2 - 8°C until expiration date. Once opened the reagents are stable for 2 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.
- Pipetting of all standards, samples, and controls should be completed within 6 minutes. (Note this especially for manual pipetting.)

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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. Sample can be stored at 2-8 °C up to 5 days. For long-term storage, aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. It is recommended that samples should only be frozen-thawed once.

Note:

Do not use haemolytic, icteric or lipaemic specimens.

Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- Standards: : Reconstitute the lyophilized contents of the standard with 1 ml distilled water and let the standards stand in the vials for few minutes at room temperature. Mix several times before use. The reconstituted standards are stable for 2 months at 2-8°C. For longer storage, aliquot and store at ≤ -20 °C.
- Sample: If the initial assay found samples contain FSH higher than the highest standard, the samples can be diluted with Standard 0 and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  a) Dilution 1:10: 10 µL Serum + 90 µL Standard 0 (mix thoroughly).

  b) Dilution 1:100: 10 µL 1:10 diluted a) + 90 µL Standard 0 (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **25 µl** of **Standard, controls and samples** in duplicate into the appropriate wells.
3. Add **100 µl** of **HRP-FSH antibody** into each well. Thoroughly mix for 10 sec. (Mixing completely is important in this step). Incubate for **30 minutes** at **RT.**
4. Aspirate each well and wash, repeating the process 4 times for a **total 5 washes**. Wash by filling each well with distilled water (**400 µl**) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µl** of **TMB substrate** to each well. Incubate for **10 minutes** at **room temperature** in dark.
6. Add **50 µl** of **Stop Solution** to each well.
7. Read the OD with a microplate reader at **450 nm** immediately. It is recommended that the wells should be read **within 10 min** after adding Stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-log linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

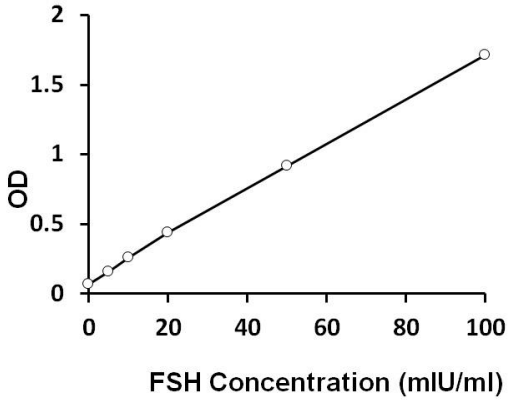
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 100 mIU/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be 0.856 mIU/ml.

Assay Range

0.86-100 mIU/ml

Specificity

The following substances were tested for cross reactivity of the assay:

Hormone Tested	Concentration	Produced Color Intensity Equivalent to FSH in serum (mIU/ml)
hCG (WHO 1st IRP75/537)	10.000 mIU/ml	0
	50.000 mIU/ml	0
	100.000 mIU/ml	0
TSH (WHO 2nd IRP 80/558)	50 µIU/ml	0
	100 µIU/ml	0
LH (WHO 1st IRP 68/40)	100 mIU/ml	0
	250 mIU/ml	0
	500 mIU/ml	0
Prolactin (WHO 1st IRP 75/504)	100 ng/ml	0
	200 ng/ml	0
hGH (WHO 1st IRP 66/217)	100 ng/ml	0
	200 ng/ml	0

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 5.53% and the CV value of inter-assay precision was 6.06%.

Interferences

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

Recovery

88.5-112.1