

---

# Human Follicle-Stimulating Hormone (FSH) ELISA kit

Catalog No.E0830h

96 Tests

Operating instruction

[USCNLIFE™](http://www.uscnlife.com)

[www.uscnlife.com](http://www.uscnlife.com)

---

**FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!**

**PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!**

## **Intended use**

This immunoassay kit allows for the specific measurement of Human Follicle-Stimulating Hormone (FSH) concentrations in serum and plasma.

## **Introduction**

Follicle stimulating hormone (FSH) is a hormone synthesised and secreted by gonadotropes in the anterior pituitary gland. In the ovary FSH stimulates the growth of immature Graafian follicles to maturation. 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 in structure; therefore the biological and immunological properties of each hormone 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, and 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.

## **Test principle**

The microtiter plate provided in this kit has been pre-coated with an antibody specific to FSH. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for FSH. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain FSH, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a

---

change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm $\pm$ 2 nm. The concentration of FSH in the samples is then determined by comparing the O.D. of the samples to the standard curve.

### Materials and components

Reagent	Quantity
Assay plate	1
Standard	2
Sample Diluent	1 x 20ml
Assay Diluent A	1 x 10ml
Assay Diluent B	1 x 10ml
Detection Reagent A	1 x 120ul
Detection Reagent B	1 x 120ul
Wash Buffer (25 x concentrate)	1 x 30ml
Substrate	1 x 10ml
Stop Solution	1 x 10ml

### 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 approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20° C.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8° C within 30 minutes of collection. Store samples at  $\leq$  -20° C. Avoid repeated freeze-thaw cycles.

**Note:** Citrate plasma has not been validated for use in this assay.

### Limitations of the procedure

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, further dilute the samples with the Assay 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.
4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

### Reagent preparation

**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into

---

deionized or distilled water to prepare 500 mL of Wash Buffer.

**Standard** - Reconstitute the **Standard** with 1.0 mL of **Sample Diluent**. This reconstitution produces a stock solution of 100 mIU/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (100 mIU/mL). The **Sample Diluent** serves as the zero standard (0 mIU/mL).

**Detection Reagent A and B** - Dilute to the working concentration specified on the vial label using **Assay Diluent A and B** (1:100), respectively.

### **Assay procedure**

Allow all reagents to reach room temperature. Arrange and label required number of strips.

1. Prepare all reagents, working standards and samples as directed in the previous sections.
2. Add 100 uL of **Standard**, Control, or sample\* per well. Cover with the adhesive strip. Incubate for 2 hours at 37° C.
3. Remove the liquid of each well, don't wash.
4. Add 100 uL of **Detection Reagent A** to each well. Incubate for 1 hour at 37°C. **Detection Reagent A** may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
5. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 uL) 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.
6. Add 100 uL of **Detection Reagent B** to each well. Cover with a new adhesive strip. Incubate for 1 hours at 37° C.
7. Repeat the aspiration/wash as in step 5.
8. Add 90 uL of **Substrate Solution** to each well. Incubate for 30 minutes at room temperature. Protect from light.
9. Add 50 uL of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

### **Specificity**

This assay recognizes recombinant and natural Human FSH. No significant cross-reactivity or interference was observed.

### **Sensitivity**

The minimum detectable dose of Human FSH is typically less than 0.78 mIU/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

### **Detection Range**

1.56-100 mIU/ml. The assay range was estimated by calculating the coefficient of variation (CV) of each standard constructing five independent standard curves. The standard curve concentrations used for the ELISA's were 100 mIU/ml, 50 mIU/ml, 25 mIU/ml, 12.5 mIU/ml, 6.2 mIU/ml, 3.1 mIU/ml, 1.56 mIU/ml, .

---

**Important Note:**

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.
4. When mixing or reconstituting protein solutions, always avoid foaming.
5. 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.
6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

**Calculation of results**

Average the duplicate readings for each standard, control, 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 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 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.

**Storage of test kits and instrumentation**

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). 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 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

**Precaution**

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.