

## Product Information

### FSH Ultra Sensitive CLIA

Catalog Number **SE120058**  
Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

Follicle-Stimulating Hormone (FSH) is a glycoprotein produced by the anterior pituitary gland. 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 follicular growth, prepares ovarian follicles for action by LH, and enhances the LH induced release of estrogen. FSH levels are elevated after menopause, castration, and in premature ovarian failure. Although there are significant exceptions ovarian failure is indicated when random FSH concentrations exceed 40 mIU/ml. In the male, FSH stimulates seminiferous tubule and testicular growth and is involved in the early stages of spermatogenesis. Oligospermic males usually have elevated FSH levels. Tumors of the testes generally depress serum FSH concentrations, but levels of LH are elevated. 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. In children, abnormalities in concentration of FSH can be aid in the diagnosis of pituitary disorders, and may be indicative of problems in the reproductive system of both genders, infertility problems, early and delay puberty.

The FSH Ultra Sensitive CLIA (Chemiluminescence Enzyme Linked Immunosorbent Assay) kit is used for the quantitative measurement of FSH in human serum or plasma.

The FSH is a solid phase direct sandwich Chemiluminescence ELISA method. The samples and diluted anti-FSH-HRP conjugate are added to the wells coated with MAb to FSH beta subunit. FSH in the serum binds to anti-FSH MAb on the well and the anti-FSH-HRP second antibody then binds to FSH. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the enzyme activities are proportional to the concentration of FSH in the samples. A standard curve is prepared relating light units to the concentration of the FSH.

### Components

Materials Provided	96 Tests
Microwell coated with FSH MAb	6 × 8 × 2
FSH Standard: 7 vials (ready to use)	0.2 ml
Enzyme Conjugate, 20×: 1 vial	0.7 ml
Assay Diluent: 1 bottle (ready to use)	12 ml
Luminol Substrate, 3×: 1 bottle	4 ml
Luminol Buffer: 1 bottle	8 ml
Wash Concentrate, 20×: 1 bottle	25 ml

### Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- Microplate luminometer
- Absorbent paper or paper towel
- Graph paper

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Preparation Instructions

### Sample Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at 2–8 °C for 5 days. If storage time exceeds 5 days, store frozen at –20 °C for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

**Note:** Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

### Reagent preparation

- **20× Enzyme Conjugate:** Prepare 1× working dilution by diluting 20-fold with assay diluent as needed (e.g., 0.1 ml of the 20× Enzyme Conjugate in 1.9 ml of assay diluent is sufficient for 20 wells). The diluted conjugate has to be used the same day.
- **20× Wash Buffer Concentrate:** Prepare 1× wash buffer by adding the contents of the bottle to 475 ml of distilled water. Store 1× wash buffer at room temperature.
- **3× Luminol Substrate:** Prepare 1× Luminol Substrate solution by adding 1 part of 3× Luminol Substrate to 2 parts Luminol Buffer as needed.

### Storage/Stability

Store the kit at 2–8 °C.

### Procedure

**Notes:** The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, controls, and serum samples be run in duplicate

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Bring all specimens and kit reagents to room temperature (18–26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Add 25 µl of FSH standards, control, and sera into selected wells.
3. Add 100 µl of 1× Enzyme Conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18–26 °C) with shaking.
5. Remove liquid from all wells. Wash wells 5 times with 300 µl of 1× wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of 1× Luminol Substrate solution to all wells.
7. Read the relative light units (RLU) in each well using a luminometer (0.2–1 second integration time) within 5 minutes of substrate addition.  
**Note:** Loss of sensitivity in low range standards may be observed if the wait time is more than 10 minutes after adding the substrate

## Results

### Calculations

The standard curve is constructed as follows:

1. Check FSH standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the RLU (Relative Light Units) for each FSH standard point (vertical axis) versus the FSH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

Example of Standard Curve

	RLU	Concentration mIU/ml
<b>Std 1</b>	7,883	0
<b>Std 2</b>	33,396	0.05
<b>Std 3</b>	130,666	0.2
<b>Std 4</b>	760,970	1
<b>Std 5</b>	1,541,193	2
<b>Std 6</b>	8,045,890	10
<b>Std 7</b>	32,608,776	40

3. Read the concentration for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

### Expected Values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:

	Male (mIU/ml)	Female (mIU/ml)
<b>Adult</b>	1.48–14.26	Follicular phase: 1.37–9.90
		Mid-cycle: 6.17–17.20
		Luteal phase: 1.09–9.20
		Post-menopausal: 14.9–124.30
<b>Children</b>		
2 weeks	1.22–5.19	2.09–30.45
1–18 months	0.19–2.97	1.14–14.35
19 months – 7 y	0.25–1.92	0.70–3.39
8–9 y	0.30–1.67	0.28–5.64
10–11 y	0.20–5.79	0.68–7.26
12–14 y	0.23–10.3	1.02–9.24
15–18 y	0.81–8.18	0.33–10.54

**Note:** The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings, and other diagnostic procedures.

### Product Profile

#### Correlation with a Reference ELISA kit

A total of 89 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.95	0.91	0.2

#### Precision

Serum	Number of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	16	0.12	0.014	11.6
2	16	0.363	0.023	6.3
3	16	0.769	0.04	5.2

#### Sensitivity

The sensitivity was determined by calculating the mean plus 2 SD of the standard zero point tested 20 times in the same run.

Serum	Number of Replicates	Mean mIU/ml	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.003	0.0014	0.0058 mIU/ml

#### Recovery

Known quantities of FSH were added to a serum that contained a low concentration of FSH.

Expected Value (mIU/ml)	Recovered (mIU/ml)	% of Recovery
2.5	2.3	92
10	11	110
20	19.2	96

#### Linearity

Two different patient samples were diluted with the "0" calibrator to 1:2, 1:4, and 1:8. FSH values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Serum	Original Value (mIU/ml)	Percentage of Recovery		
		1:2	1:4	1:8
1	2	96	101	93
2	5	94	97	102

**References**

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