

FOLLICLE-STIMULATION HORMONE (FSH) ENZYME IMMUNOASSAY TEST KIT Catalog Number: 6107325



Enzyme Immunoassay for the Quantitative Determination of Follicle-Stimulation Hormone (FSH) Concentration in Human Serum

For In Vitro Diagnostic Use Only

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

FSH Enzyme Immunoassay

INTENDED USE

For the quantitative determination of follicle-stimulation hormone (FSH) concentration in human serum.

INTRODUCTION

Human follicle-stimulating hormone (FSH, follitropin) is a glycoprotein produced and secreted by the basophilic cells of the anterior lobe of the pituitary gland. Secretion of FSH is stimulated by gonadotropin-releasing hormone (GnRH). Gonadal steroids like progesterone, estrogens and androgens, exert both positive and negative feedback on FSH function. Pulse-like secretion of FSH is more pronounced in women than in men. In women, FSH levels vary during the menstrual cycle. FSH stimulates maturation of ovarian follicles at the beginning of the cycle. Ovulation is preceded by a surge in FSH (mid-cycle phase). Base levels are slightly higher at the beginning of the cycle (follicular phase) than at the end of the cycle (luteal phase). In postmenopausal women, FSH levels are significantly increased because of lack of negative feedback from ovarian steroids. In men, FSH and LH maintain spermatogenesis in the testes. FSH levels in prepubertal children are low. FSH determination is important in diagnosis in women with disturbances of the menstrual cycle, primary and secondary amenorrhea, hirsutism or virilism. FSH is a good indicator of onset of the menopause. In men, determination of FSH is useful in the diagnosis infertility, hypogonadism, gynaecomastia and tumours. In children, assessment of FSH is important in investigating delayed or precocious puberty.

PRINCIPLE OF THE TEST

The ELISA test is performed as an indirect solid phase sandwich-type immunoassay. Microwells are coated with anti-monoclonal FSH followed by blocking the unreacted sites to reduce non-specific binding.

- Step 1 FSH Antigens present in calibrators and patient samples bind to the coated antibody.
- Step 2 The Antigen-Antibody complex is reacted with enzyme (HRP) labeled anti-monoclonal FSH conjugate resulting in the FSH antigen being

sandwiched between the solid phase antibody and the enzyme conjugate.

- Step 3 The enzyme converts added substrate (TMB) to form a colored solution.
- Step 4 The intensity of color change, which is proportional to the concentration of Antibodies present in the samples is read by a microplate-reader at 450 nm. Results are expressed in mIU/ml.

REAGENTS

Materials provided with the kit:

- **Microwell plate.** 12x8 well strips, Individually separable wells. coated with anti-monoclonal FSH.
- **Calibrators.** Ready to use. 0, 5; 15; 45; and 135 mIU/mL. 5 Vials x 0.4 mL.
- **Enzyme Conjugate.** FSH HRP (Horseradish Peroxidase) conjugate. Ready to use. 12 mL.
- **Substrate solution.** H₂O₂-TMB 0.25 g/L (avoid any skin contact). 12 mL
- **Stop Solution.** Sulphuric acid 0.15 mol/L (corrosive: avoid any skin contact). 12 mL.

Materials required but not provided:

- Multichannel pipettes and micropipettes (Precision $\geq 1.5\%$) and disposable tips.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 655 nm is advisable.
- Manual or automated wash system.
- Absorbent paper of blotting the microplate wells.
- Distilled or deionised water.
- Timer.

EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population. The serum or plasma FSH values are comprised in the following intervals:

Sample	Range mIU/mL
Male	2.0 – 14.0
Female:	
Follicular phase	2.0 – 10.0
Ovulatory phase	6.0 – 24.0
Luteal phase	1.5 – 8.0
Post Menopausal	17.0 – 95.0
Pregnant female	0.0 – 11.6

Some of the female population tested in this group were probably using oral contraceptives, which may affect results.

STORAGE OF TEST KITS



FOLLICLE-STIMULATION HORMONE (FSH) ENZYME IMMUNOASSAY TEST KIT Catalog Number: 6107325



The components will remain stable through the expiration date shown on the label if stored between 2-8°C in dark. Do not freeze. Do not use reagents beyond the kit expiration date.

The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells.

Once opened the bag, microplate strips are stable for 1 month at 2-8°C in the plastic bag tightly sealed, with the silicagel.

Opened reagents are stable for 1 month at 2-8°C.

REAGENT PREPARATION

- Coated microwell strips are only for a single use.
- Calibrators, Conjugate, Substrate Solution and Stop Solution are ready to use and need not to be diluted.

Precautions

- Instructions should be followed exactly as they appear in this kit insert to ensure valid results.
- Avoid contact with the **TMB (3,3',5,5'-Tetramethylbenzidine)**. If TMB comes into contact with skin wash thoroughly with water and soap.
- The stop solution contains **sulphuric acid**. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
- Avoid contact between the buffered **peroxide** solution and easily oxidized materials; extreme temperatures may initiate spontaneous combustion.
- Do not use beyond expiration date on the label.
- Do not use if reagent is not clear or if a precipitate is present.
- Do not interchange kit components with those from other sources other than the same catalog number from LINEAR.
- Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling.
- All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests.
However, human blood derivatives and patient specimens should be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials.

SPECIMEN COLLECTION AND PREPARATION

Use fresh serum or plasma. Samples can be stored at 2-8°C for 2 days. For longer periods, samples should be frozen (-20°C). Avoid repeated freezing and thawing.

Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Neither Bilirubin nor Hemolysis have significant effect on the procedure.

Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Neither Bilirubin nor Hemolysis have significant effect on the procedure.

For sample with concentration over 135 mIU/mL dilute the sample 1/1 with dist. Water.

ASSAY PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let specimens and test reagents equilibrate at room temperature before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells.
- Return pouch immediately to refrigerator.
- Good washing technique is critical. For manual washing, fill each microwell with 250 µL distilled water. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer's instructions.
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.

Preparation of sample

Usually no dilution necessary; dilute samples with concentrations above 135 mIU/mL 1:1 with Test dist. water.

Procedure

Label protocol sheet to indicate sample placement in the wells according to the following figure. **5 calibrators** (standards) (SA-SE) and **1 Blank** should be included. The user has the option to run Patient Samples (P) in duplicate.



FOLLICLE-STIMULATION HORMONE (FSH) ENZYME IMMUNOASSAY TEST KIT Catalog Number: 6107325



	1	2	3	4	5	6	7	8	9	10	11	12
a	B	SD	P3									
b	SA	SE	P4									
c	SA	SE	P4									
d	SB	P1	P.									
e	SB	P1	P.									
f	SC	P2										
g	SC	P2										
h	SD	P3										

Cal.	Conc.
	mIU/mL
SA	0
SB	5
SC	15
SD	45
SE	135

The assay should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. This method should be used for testing human serum samples only.

PERFORMANCE CHARACTERISTICS

Sensitivity

The minimal detectable concentration of Human FSH by this assay is estimated to be 1.0 mIU/mL.

Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

hFSH	100.0 %
hLH	< 0.2 %
HCG	< 0.1 %

Precision

a. Intra Assay variation

Within-run precision was determined by replicate determination of three different control in one assay. The within assay variability is shown below:

Sample	1	2	3
Number of replicates	16	16	16
Mean FSH (mIU/ml)	6.9	19.7	51.3
Std. Deviation	0.42	1.27	3.55
Coef. of Variation (%)	6.1	6.4	6.9

b. Inter Assay variation

Between-run precision was determined by replicate determination of three different controls in one assay. The between assay variability is shown below:

Sample	1	2	3
Number of replicates	16	16	16
Mean FSH (mIU/ml)	7.3	21.1	50.3
Std. Variation	0.62	1.75	4.56
Coef. of Variation (%)	8.5	8.3	9.1

Recovery

Various patient samples of known FSH levels were combined and assayed in duplicate. The average recovery 103.1% with reference to the original concentrations.

Expected conc.	Observed conc.	Recovery
6.6	7.1	107.5
12.2	11.7	95.9
41.8	44.5	106.4
38.3	37.4	97.7
78.1	84.3	107.9

Linearity

Two patient samples were serially diluted with zero standard in a linearity study. The average recovery was 101.2 %.

Patient	Exp. Conc	Obs. Conc	Recovery
1		66.8	

1. Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder .
2. Pipette 25 µL of Calibrators and Patient Samples into the wells and incubate 15 minutes at room temperature
3. Add 100 µL of Enzyme Conjugate to the wells except for Blank well and incubate 30 minutes at room temperature.
4. Add app. 300 µL of distilled water, decant (tap or blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes.
5. Pipette 100 µL of Substrate Solution into each microwell in the same order and timing as for the Enzyme Conjugate, Blank well included.
6. Incubate 10 minutes at room temperature in the dark.
7. Add 100 µL of Stop Solution into each microwell using the same order and timing as for the addition of the Substrate Solution.
8. Read absorbance of each microwell at 450 nm against blank using a microplate reader. The developed color is stable for at least 30 minutes. Read optical densities during this time.

TEST EVALUATION

Mean absorbance and relative percentage

1. Calculate the mean of the absorbances (Em) corresponding to the single points to the standard curve and of each sample.
2. Subtract the mean absorbance value of the zero standard from the mean absorbance values of standards and samples.
3. Draw the standard curve on log-log or lin-lin graph paper by plotting absorbance values of standard against appropriate FSH concentration.
4. Read off the FSH concentrations of the samples.

LIMITATIONS OF THE PROCEDURE



FOLLICLE-STIMULATION HORMONE (FSH) ENZYME IMMUNOASSAY TEST KIT Catalog Number: 6107325



Dil. 1 / 2	33.4	34.6	103.6
Dil. 1 / 4	16.7	18.1	108.3
Dil. 1 / 8	8.3	7.7	92.8

2		120.4	
Dil. 1 / 2	60.2	58.3	98.0
Dil. 1 / 4	30.1	29.0	96.3
Dil. 1 / 8	15.0	15.9	108.0

Limitations of the procedure assay, no hook effect is observed up to 4000 mIU/mL of FSH.

REFERENCES

1. Wisdom, G.B. Clin. Chem. 22/8 1243 - 1255 (1976)
2. Shome, B. and Parlow, A.F., J. Clin. Endocr. Metab.,39, 199 – 202 (1974).
3. Shome, B. and Parlow, A.F., J. Clin. Endocr. Metab.,39, 203 – 205 (1974).
4. Uotila, M. Ruoslahti, E. Engvall, E., J. Immunol. Methds, 42, 11-15 (1981)
5. Acosta, A.A.M.D. and Wright, G.L., Journal of Clinical Immunoassays, 6, 41 (1983).
6. Cohen, K. L., Metabolism, 26 1165-1177 (1977)

6107325-3/0809
R2.ing

