



GonadotropinEIA-FSH

Enzyme-linked Immunosorbent Assay for Quantitative Determination of Follicle-Stimulating Hormone in Human Serum

1. INTENDED USE

1.1. This kit is intended for the quantitative determination of follicle-stimulating hormone (FSH) in human serum.

1.2. FSH is a glycoprotein with a molecular weight of about 30000 Da, that consists of two subunits – alpha and beta. FSH is secreted by frontal lobe of pituitary gland.

Together with LH and testosterone, FSH is necessary for spermatogenesis in spermatic ducts of testicles. In pubescent females FSH induces follicle growth and maturation in ovaries. Increased FSH levels are observed in patients with different forms of hypogonadism (primary ovary or testicle insufficiency, polycystic ovaries, menopause etc), renal insufficiency, cirrhosis and also as a result of castration. As a rule, decreased FSH levels are observed in the case of testicle malignancies.

FSH measurement is useful for the diagnosis of menopause, exact determination of ovulation time and for endocrine therapy monitoring.

2. ASSAY DESCRIPTION AND PRINCIPLES.

2.1. Kit Contents:

- microtitration strips, 12x8 wells, coated with anti-FSH monoclonal antibodies, packed in the plastic bag, labeled "Strips with monoclonal antibodies against FSH", 1 bag;
- FSH calibrators (protein-based buffer containing known FSH concentrations). Calibrators have been standardized against the WHO 2nd International Reference Preparation 78/549. Exact FSH concentrations are indicated on vial labels, 6 vials, 0,5 ml each;
- anti-FSH antibodies conjugated with horseradish peroxidase, labeled "Conjugate", 1 vial, 14 ml;
- concentrated wash solution, labeled "Buffer", 1 vial, 20 ml;
- tetramethylbenzidine substrate solution, labeled "TMB solution", 1 vial, 14 ml;
- "Stop reagent", 1 vial, 14 ml;
- control (protein-based buffer containing known FSH concentration), labeled "Control", 1 vial, 0,5 ml.

2.2. "GonadotropinEIA-FSH" reagents are sufficient for determination of 40 unknowns, 6 calibrators, 1 control and 1 TMB Substrate control in duplicates, providing that all the strips are used simultaneously.

NOTE: If used partially, kit should be used within a month after opening.

2.3. Assay principle. “GonadotropinEIA-FSH” is a “sandwich” type of solid-phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of FSH molecule. One of these antibodies (anti- α -subunit) is conjugated with horseradish peroxidase; the other (anti- β -subunit) is immobilized on inner surface of microwells. During the incubation FSH molecules from the serum sample bind to both immobilized antibodies and anti-FSH-peroxidase conjugate. Then the wells are washed with wash buffer to remove any material not bound to the inner surface of the wells. The quantity of the bound conjugate is in direct proportion to the FSH concentration in tested sample. During the incubation with TMB Substrate solution the coloring appears. The color intensity is in direct proportion to the FSH concentration in sample. Optical density of the solution in the wells is measured and the FSH concentration in the samples is calculated using calibration curve.

3. PERFORMANCE CHARACTERISTICS

3.1. Specificity. No cross-interaction of monoclonal antibodies against beta-FSH with LH, TSH and hCG was detected.

3.2. Coefficient of variation (intra-assay precision) between the results of FSH determination in the same sample is less than 8%.

3.3. Linearity (Dilution test). Dilution of serum sample containing predetermined FSH concentration with FSH-free serum leads to linear recovery of FSH in diluted samples in the concentration range between Calibrator №2 and Calibrator №6.

3.4. Recovery. To determine this parameter, equal volumes of control and Calibrator №3 were mixed. Then the correspondence between the calculated FSH concentration in the obtained sample and the measured concentration was determined. Recovery range is 90–110%.

3.5. Detectability. Minimal FSH concentration detectable by “GonadotropinEIA-FSH” assay is 0.3 mIU/ml.

3.6. Expected values. Serum samples collected between 9 and 11 a.m. from apparently healthy people, both males and females, at the age of 19–65, were assayed with “GonadotropinEIA-FSH” test kit (see Table1 for results). These limits should be considered as guidelines only.

Table 1.

Category	N	Mean (mIU/ml)	Range (mIU/ml)
<i>Females</i>			
Normally menstruating (19-35 years old)	120		
Follicular phase		4.6	1.8-11.3
Midcycle peak		7.9	4.9-20.4
Luteal phase		3.3	1.1-9.5
Postmenopausal (49-65 years old)	15	68.4	31.0-130
<i>Males (21-39 years old)</i>	40	3.9	1.0-11.8

3.7. It is highly recommended for each laboratory to determine its own reference range of FSH concentrations.

4. WARNINGS AND PRECAUTIONS

4.1. All the components are non-toxic.

4.2. Stop reagent is 1N HCl solution. Avoid contacts with skin and mucosa. In case of contact rinse affected region thoroughly with plenty of water.

4.3. It is highly recommended to handle kit components in accordance with established good laboratory practice. The operator should wear disposable latex or plastic gloves and handle patients samples as if capable of transmitting infectious agents.

5. MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT:

- digital variable pipettes that cover volume ranges from 0.005 to 0.05 ml; from 0.04 to 0.2 ml; from 0.2 to 1 ml and from 1 to 5 ml, with appropriate disposable tips;

- 8-channel digital variable pipette that covers volume range up to 0.3 ml, with appropriate disposable tips;

- microplate shaker-thermostat, able to maintain temperature +37°C and shaking speed 500 to 800 rpm;;

- automatic microplate reader;

- 200 ml volumetric cylinder;

- 300 ml volumetric beaker;

- distilled water.

6. REAGENT PREPARATION FOR ASSAY

6.1. FSH calibrators and control are ready to use. Once opened, store at +2... 8°C for no more than 1 month.

6.2. Microtitration strips. Before opening keep the bag at room temperature (+18...25°C) for 30 minutes. Open the bag and place required number of strips on strip holder. Put remaining strips back in plastic bag and close tightly. Keep at +2...8°C until expiry date stated on the label.

6.3 Wash buffer. Prepare the necessary volume of Wash buffer by dilution of Buffer P 10-fold with distilled water. For example:

5 ml of Buffer P + 45 ml of distilled water. Mix thoroughly, avoiding foaming.

Keep firmly closed. Store at room temperature (+18...25°C) for no more than 5 days. The rest of the Buffer P should be stored firmly closed at +2...8°C until expiry date.

6.4. Conjugate E is ready to use. Once opened, store at +2...8°C for no more than 1 month.

6.5. TMB Substrate solution is ready to use. Once opened, store at +2...8°C for no more than 1 month.

6.6. Stop reagent is ready to use. Once opened, store at +2...8°C until expiry date.

7. ASSAY PROCEDURE

7.1. All the kit components and serum samples should be brought to room temperature (+18...25°C) and stirred thoroughly before the assay. Assay scheme is given on the last page.

7.2. Mark the wells as follows:

A1, A2 — № 1 for TMB substrate control;
B1, B2 — № 2 for calibrator № 1;
C1, C2 — № 3 for calibrator № 2;
D1, D2 — № 4 for calibrator № 3;
E1, E2 — № 5 for calibrator № 4;
F1, F2 — № 6 for calibrator № 5;
G1, G2 — № 7 for calibrator № 6;
H1, H2 — № 8 for control.

7.3. Perform each assay in duplicate for both calibrators and unknowns.

7.4. Pipette 100 µl of Conjugate E into each well **except A1 and A2 wells**.

7.5. Pipette 50 µl of FSH calibrators, control and serum samples into the corresponding wells.

7.6. Incubate strips for 1 hour on shaker-thermostat at +37°C (500-800 rpm).

7.7. After incubation, aspirate the liquid from the wells and wash the strips five times with wash buffer. Each time add 300 µl of wash buffer per well and shake for 5–10 sec, then aspirate the buffer. After the last washing cycle, if necessary, invert the plate and firmly tap on a clean paper towel to remove remaining wash buffer.

7.8. Add 100 µl of TMB Substrate solution into each well. Incubate at room temperature in the dark for 10 – 30 min depending on the color intensity.

7.9. Add 100 µl of Stop reagent to all wells and shake well for 1–2 minutes.

7.10. Read the optical density of the solution in the microwells at a wavelength of 450 nm.

7.11. Calculate the mean absorbency for each duplicate. Subtract the mean absorbency value of A1-A2 wells from the mean absorbency values of calibrators; control and unknown samples. Draw the calibration curve in linear coordinates by plotting absorbency values for calibrators against corresponding FSH concentrations. Determine FSH concentrations in the unknown samples and control.

7.12. An appropriate computer software may be used for calculations. Use the mean absorbency value of A1-A2 wells as “blank”.

8. PROCEDURAL NOTES

8.1. “GonadotropinEIA-FSH” kit should be stored at + 2...8°C until expiry date stated on the label.

If used for separate experiments kit contents should be stored as follows:

- put remaining strips in plastic bag and close tightly. Keep at +2...8°C until expiry date;
- once opened, store Conjugate E and TMB substrate solution at + 2...8°C for no more than 1 month;
- once opened, store Buffer P at +2...8°C until expiry date;
- store prepared wash buffer firmly closed at room temperature for no more than 5 days;

- store calibrators and control at +2...+8°C for no more than 1 month after opening;

- store Stop reagent at +2...8°C until expiry date.

8.2. Do not use plasma, hemolysed or lipemic serum or samples with sodium azide as a preservative.

8.3. Please take into consideration that calibrators should be measured in each separate assay. It is also recommended to measure FSH concentration in the control each time. The number of separate experiments that can be performed with one kit (4 experiments) is therefore limited by the volume of calibrators.

8.4. Do not use Stop reagents from other manufacturers.

8.5. The operator should thoroughly follow the manual to obtain the reliable result.

SCHEME OF ASSAY

Stage of assay and reagents used	Numeration of the wells (according to 7.2)								
	1	2	3	4	5	6	7	8	9–48
Conjugate E, µl	–	100	100	100	100	100	100	100	100
Calibrator № 1, µl	–	50	–	–	–	–	–	–	–
Calibrator № 2, µl	–	–	50	–	–	–	–	–	–
Calibrator № 3, µl	–	–	–	50	–	–	–	–	–
Calibrator № 4, µl	–	–	–	–	50	–	–	–	–
Calibrator № 5, µl	–	–	–	–	–	50	–	–	–
Calibrator № 6, µl	–	–	–	–	–	–	50	–	–
Control, µl	–	–	–	–	–	–	–	50	–
Unknown sample, µl	–	–	–	–	–	–	–	–	50
Incubation № 1	1 hour on shaker-thermostat at +37°C								
Wash (five times): wash buffer, µl	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300
TMB solution, µl	100	100	100	100	100	100	100	100	100
Incubation № 2	15-30 minutes in the dark at room temperature								
Stop Reagent, µl	100	100	100	100	100	100	100	100	100
Stirring	1-2 minutes on shaker								
OD measuring	Microplate reader (wavelength 450 nm)								
Calculations	Corresponding software (recommended)								

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