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PADTAN ELM

THE PADTAN ELM EIA KIT FOR 96 TESTS

FSH EIA Kit

Intended use:
Quantitative determination of FSH levels in human serum and plasma.

For *in vitro* diagnostic use.

I. Introduction

Follicle Stimulating hormone (FSH) or follitropin, secreted by the gonadotropin cells of anterior pituitary gland, is a glycoprotein hormone with a molecular weight of 30 kDa.
FSH contains two different subunits (α and β) linked by noncovalent bounds. The α subunit, containing 89 amino-acid residues, shares structural homology with other hormones: Luteinizing hormone (LH), human chorionic gonadotropin (HCG), and thyroid-stimulating hormone (TSH). Thus, the α subunits of these hormones are interchangeable and devoid of biological activity. The β subunit, which displays greater differences in amino acid sequences among the various hormones, confer hormonal and immunological specificity[1-3].
FSH, stimulated by a hypothalamic decapeptide - Gonadotropin releasing hormone (GnRH) is required for normal sexual function in both women and men[4-5].
In women, FSH binds to specific cell membrane receptors on ovarian granulosa cells. This interaction includes growth and maturation of ovarian follicles.
In men, after binding to testicular Sertoli cells, FSH stimulates production of inhibin and androgen binding protein and together with LH and testosterone stimulates spermato-genesis.
The secretory patterns of FSH are very different for two sexes:
In women, during the first part of the cycle, FSH secretion is suppressed by negative feedback from steroid hormones. In response to falling hormone levels, GnRH is secreted. The latter, stimulates secretion of FSH, and LH resulting the surge of FSH.
This surge is followed by rupture of graafian follicle and ovulation. Following ovulation, in luteal phase, FSH production is suppressed by negative feedback from progesterone and estradiol[6-7].
In menopausal women, due to low levels of circulating estradiol and progesterone the negative feedback of these steroid hormones is suppressed; as a result circulating levels of FSH are greatly increased.
Measurement of FSH concentration in serum or plasma provides useful information about disorders of the hypo-thalamic- pituitary - gonadal axis and helps to differentiate among the various disorders in which the FSH concentration increases or decreases[8-10].

II. Principle of the test

The FSH quantitative test kit is based on solid phase enzyme immunoassay (EIA). This assay system uses two mouse monoclonal antibodies directed against distinct antigenic determinants on the FSH molecule. The polystyrene wells are coated with captured antibody against FSH. Standards, controls and patient samples are added to the wells (solide phase) and incubate. The FSH present in the wells is bound to the anti-FSH antibodies. The unbound material is removed by aspiration and washing. After washing, the HRP labeled anti-FSH Mab is added to the wells. After second incubation and washing, a solution of TMB (3,3',5,5' tetra-methylbenzidine) is added to each well, resulting in the development of a blue color. The intensity of the color is proportional to the amount of FSH present in the sample. The color development is stopped by addition of Stop solution, causing the blue color to change to yellow. The color intensity is determined in a microtiter plate spectrophotometer at 450 nm. Standard curves are constructed for each assay by plotting absorbance value against the concentration of each standard. The FSH concentrations of patient samples are then read from the standard curve.

III. Kit contents

The reagents provided with the FSH Kits(Cat.No.P-FSI) are sufficient for 96
wells. The expiry date of each reagent is shown on the vial label.

Store the Kit at 2-8° C.

1.Coated microtiter wells: 96 wells, coated with mouse monoclonal anti-FSH antibodies.

2.Zero standard: 1 vial (4 ml). The zero standard should also be used as sample diluents.

3.Standards: 5 vials (1.0 ml) of Human FSH in serum matrix with thimerosal as a preservative. The exact FSH concentration for each standard is specified on the label of each vial. The standards supplied with the kit were calibrated against the international standard 2nd.IRP 78/549.

4.Serum controls: 2 vials (1.0 ml) of human FSH in processed human serum containing thimerosal. The nominal FSH concentrations for each control is specified on the label of each vial.

5.Enzyme tracer: 1 vial (12 ml) of monoclonal anti-FSH antibody conjugated to horseradish peroxidase (HRP) in phosphate buffered saline (PBS) with proteins and thimerosal.

6.Wash solution (concentrated 20X): 1 vial (12 ml) of PBS-Tween 20 and thimerosal.

7.TMB HRP-Substrate: 1 vial (12 ml) of buffered H_2O_2 and 3,3',5,5' tetramethyl benzidine.

8.Stop solution: 1 vial (12ml) of 2N H_2SO_4.

IV. Materials required (but not supplied with the kit)

1.Microtiter plate spectrophotometer reader with a wave-length of 450 nm (with reference wavelength at 630 nm) and an absorbance range of 0 to 3.0.

2.Precision micropipetes to deliver 25 and 100 µl.

3. Distilled or deionized water for preparation of diluted Wash Solution.

V. Specimen collection and preparation

The assay can be performed on serum or heparinized plasma samples. Keep samples at 2-8°C for 1-2 weeks; for longer periods it is recommended to store the sample in aliquot form at -20° C. Avoid repeated freezing and thawing of samples. Prior to assay, frozen specimens should be slowly brought to room temperature and gently mixed by hand. Do not vortex patient samples. Serum sample with FSH greater than the last standard, should be diluted with "zero" standard and reassayed to give a quantitative result. The value obtained must be multiplied by the dilution factor to give the correct FSH concentration.

VI. Assay procedures

All reagents should be brought to room temperature prior to use.

Concentrated wash solution must be diluted with distilled or deionizer water (Dilute one part concentrated wash solution with 19 parts of water). Diluted wash solution is stable for 7 days at 2 to 8°C. In presence of undissolved crystals, resuspend the solution by placing the vial at 37° C for a few minutes.

1.Dispense 25 µl of FSH standards, control serums and patient samples into appropriate wells. Pipette 100 µl of HRP anti-FSH to each well and thoroughly mix the plate for 15 seconds.

2.Incubate the strips for 1 hour at room temperature.

3.After incubation time, aspirate and wash each well 4 times with 300 µl of diluted wash solution.

4.Dispense 100 µl of TMB HRP-Substrate into each well.

5.Incubate at room temperature in the dark for 10 minutes.

6. Add 100 µl of Stop Solution and mix for 10 seconds. Read absorbance at 450 nm (with reference wavelength at 630 nm) in a microtiter plate reader within 15 minutes after addition of stop solution

SUMMARY OF ASSAY PROCEDURE

Pipette 25 µl standard,control or sample

Pipette 100 µl Anti-FSH HRP

Incubate 1 hour at RT

Wash 4 x(300 µl)

Pipette 100 µl TMB

Incubate 10 min. at RT

Pipette 100 µl Stop Solution

Read at 450/630 nm

VII. Calculation of results

The results can be calculated by either microtiter plate, spectrophotometer reader or manual evaluation. If a microtiter plate spectrophotometer reader with data calculation program is used, refer to the plate reader and create a program using the concentration of each of the FSH standards in mIU/ml.

For manual evaluation, a standard curve is constructed by plotting the absorbance values obtained for each FSH standard against the corresponding FSH concentrations. The unknown FSH concentration, in mIU/ml, can then be read from the standard curve using the absorbance value of each patient specimen.

The standard curve must be constructed by using the corresponding FSH concentration specified on the label of each vial.

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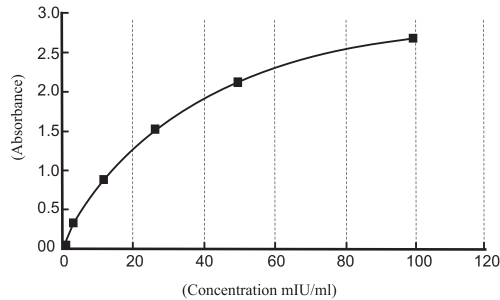
Example of calculation:

The values shown below are examples and must not be used in place of experimental data.

Specimen	Absorbance	FSH Concentration(mIU/ml)
Standard A	0.04	0.0
Standard B	0.3	2.5
Standard C	0.85	10
Standard D	1.56	25
Standard E	2.22	50
Standard F	2.77	100
Sample	1.53	24.8



VIII. Normal values

In the literature, the serum or plasma FSH concentration in healthy adults is reported as below:

Adult male:	1.5-12.4 mIU/ml
Adult female:	
- Follicular	3.5-12.5 mIU/ml
- Ovulatory Peak	4.7-21.5 mIU/ml
- Luteal	1.7-7.7 mIU/ml
- Posmenopausal	25.8-134.8 mIU/ml

We suggest that each laboratory establish its own normal ranges based on patient population.

IX. Specific assay characteristics

1.Sensitivity: The sensitivity of this kit was calculated based upon the standard curve and expressed as the minimal concentration of FSH showing a significant difference from the "zero" standard. This minimal detectable concentration of FSH is 0.5 mIU/mL

2.Specificity: The specificity of the FSH EIA test was determined by measuring the apparent FSH response caused by high levels of LH (500mIUm/L), TSH (100mIU/mL), and βHCG (100,000 mIU/mL).

The results of these cross reaction tests are shown in the following table:

Hormone		FSH (mIU/ml)	
Name	Concentration (mIU/ml)	Expected	Measured
LH	500	14.9	16.0
TSH	100	14.9	16.4
BHCG	100,000	14.9	15.4

3.Hook effect: Samples with very high antigen concentration are tested undiluted. With this kit, no hook effect has been noticed with samples containing FSH concentration up to 100,000 mIU/ml.

4.Comparison with FSH electrochemiluminescence method: Correlation studies on more than 500 random serum samples, were performed using the quantitative results from the ROCHE electrochemiluminescence (Elecsys). The correlation coefficient of test was 0.97

5. Precision: Precision was evaluated for intra- and inter-assay variability.

Intra-assay reproducibility was determined by measurement of 10 replicates of 4 samples in a single run.

Inter-assay reproducibility was determined by replicate measurement of 4 samples in 10 separate runs.

Reproducibility

	Intra-assay			Inter-assay		
	Mean(mIU/ml)	SD	%CV	Mean(mIU/ml)	SD	%CV
Sample 1	7.2	0.1	1.8	7.3	0.4	6.0
Sample 2	26.0	1.3	4.9	24.0	0.9	3.6
Sample 3	51.4	0.9	1.8	53.3	1.0	2.0
Sample 4	87.4	2.7	3.2	84.6	2.1	2.4

6. Recovery and dilution: Recovery is defined as the increase in concentration seen when a known concentration of an analyte is added to a sample. Spiked samples were prepared by mixing the appropriate aliquot of a concentrated FSH solution with a serum sample containing a low FSH concentration. The percentage recovery is calculated as:

[(Measured conc. (mIU/ml)/ Expected conc. (mIU/ml)] X100

The percentage recovery ranged from 100.0 to 108.2

Recovery Test

Added Conc. (mIU/ml)	Expected Conc. (mIU/ml)	Measured Conc. (mIU/ml)	% Recovery
-	-	14.7	-
12.9	27.6	27.6	100.0
23.3	38.0	38.5	101.3
38.8	53.5	57.9	108.2
58.2	72.9	76.4	104.8

For dilution test, several serum samples containing elevated FSH concentrations were tested after serially diluting with the zero standard. The results of one of these samples are shown in the following table. The percentage recovery ranged from 90.6-100.3.

Dilution test

Dilution	Expected Con. (mIU/ml)	Measured Con. (mIU/ml)	% Recovery
-	-	135.7	-
1:2	67.9	68.1	100.3
1:4	33.9	31.3	92.3
1:8	17.0	15.4	90.6
1:16	8.5	8.0	94.1

X. References

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WARNING!

This kit contains toxic materials, animal and human sourced components. Since no method can completely rule out the presence of blood-borne disease(e.g. HIV,HCV and HBV) therefore,all human sourced material must be considered potentially infectious.
In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary. Never pipette by month. Do not eat, smoke or apply cosmetics in the laboratory.

Rev: Jul.2015

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