



DCM010-12
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FSH ELISA

for routine analysis

Direct immunoenzymatic determination of the Follicle-Stimulating Hormon (FSH) in human serum or plasma.

IVD



LOT

See external label

2°C 8°C



Σ = 96 tests

REF DKO010

INTENDED USE

Eagle Biosciences [FSH ELISA Assay Kit](#) is a direct solid phase immunoassay for the quantitative determination of Follicle-Stimulating Hormon (FSH) in human serum or plasma.

FSH ELISA Assay Kit s intended for research use only and not toe be used in diagnostic procedures.

1. SUMMARY AND EXPLANATION OF THE TEST

Follicle Stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,500 daltons. The α- subunit is similar to other pituitary hormones [luteinizing stimulating hormone (LH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (hCG)] while the β-subunit is unique. The β- subunit confers the biological activity to the molecule. Stimulation by gonadotropin-releasing hormone (GnRH) causes release of FSH, as well as LH, from the pituitary and is transported by the blood to their sites of action, the testes or ovary.

In men, FSH acts on the Sertoli cells of the testis, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly supports spermatogenesis.

In women, FSH acts on the granulosa cells of the ovary, stimulatn steroidogenesis. All ovulatory menstrual cycles have a characteristic pattern of FSH, as well as LH, secretion. The menstrual cycle is divided into a follicular phase and a luteal phase by the midcycle surge of the gonadotropins (LH and FSH). As the follicular phase progresses, FSH concentration decreases. Near the time ovulation occur, about midcycle, FSH peaks (lesser in magnitude than LH) to its highest level.

The clinical usefulness of the measurement of Follicle Stimulating hormone (FSH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established (1,2).

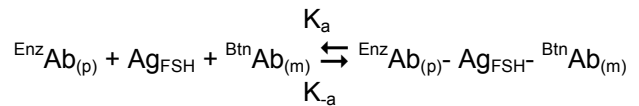
2. PRINCIPLE OF THE TEST

In the FSH ELISA Assay Kit, the essential reagents required for an immunoenzymatic assay include high affinity and specificity antibodies (enzyme-linked and

immobilised) with different and distinct epitope recognition, in excess, and native antigen.

In this procedure the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti FSH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen, reation results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:



$\text{B}^{\text{tn}}\text{Ab}_{(m)}$ = biotinylated monoclonal antibody (Excess quantity)

Ag_{FSH} = native FSH antigen (variable quantity)

$\text{EnzAb}_{(p)}$ = enzyme labeled policlonal antibody (Excess quantity)

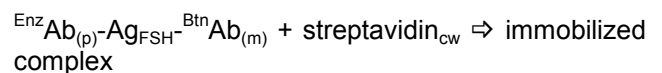
$\text{EnzAb}_{(p)} - \text{Ag}_{\text{FSH}} - \text{B}^{\text{tn}}\text{Ab}_{(m)}$ = antigen-antibodies sandwich complex

K_a = rate constant of association

K_{-a} = rate constant of disassociation

Simultaneously the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

This interaction is illustrated below:



$\text{Streptavidin}_{\text{cw}}$ = streptavidin immobilized on well

Immobilized complex = antibodies-antigen sandwich bound.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by a washing step.

The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration.

By using several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 Reagents and Materials Supplied in the Kit

1. FSH Calibrators (6 vials, 1 mL each)

CAL0	REF DCE002/1006-0
CAL1	REF DCE002/1007-0
CAL2	REF DCE002/1008-0
CAL3	REF DCE002/1009-0
CAL4	REF DCE002/1010-0
CAL5	REF DCE002/1011-0

2. FSH Control (1 vial, 1 mL)

Control concentration is Lot-specific and it is indicated on the Certificate of Analysis

REF DCE045/1003-0

3. Conjugate (1 vial, 12 mL)

Antibody anti FSH conjugated with Horseradish peroxidase (HRP)

Antibody anti FSH conjugated with Biotine

REF DCE002/1002-0

4. Coated Microplate (1 breakable microplate)

Streptavidin adsorbed on microplate

REF DCE002/1003-0

5. TMB Substrate (1 vial, 15 mL)

H₂O₂-TMB 0.26 g /L (avoid any skin contact)

REF DCE004-0

6. Stop Solution (1 vial, 15 mL)

Sulphuric acid 0.15 mol/L (avoid any skin contact)

REF DCE005-0

7. 10X Conc. Wash Solution (1 vial, 50 mL)

Phosphate buffer 0.2M

REF DCE054-0

3.2 Reagents necessary not supplied

Distilled water.

3.3 Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (450 nm)

Note

Store all reagents at 2-8°C in the dark.

Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable up to expiry date of the kit.

4. WARNINGS

- This FSH ELISA Assay Kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.

- Some reagents of the FSH ELISA Assay Kit contain small amounts of Proclin 300^R as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants. Do not freeze the solution.
- This FSH ELISA Assay Kit allows the determination of FSH from 5 to 100 mIU/mL.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents of the FSH ELISA Assay Kit should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all FSH ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange FSH ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of reagents.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.

- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C₀...C₅)

The Calibrators are ready to use, are calibrated against the International Standard WHO 2nd IRP 78/549 and have the following concentration:

	C ₀	C ₁	C ₂	C ₃	C ₄	C ₅
mIU/mL	0	5	10	25	50	100

For sample with concentration over 100 mIU/mL dilute the sample 1:2 with the Calibrator 0.

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of the Wash Solution

Dilute the content of each vial of the "10X Conc. Wash Solution" with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

In concentrated wash solution is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals; for greater accuracy dilute the whole content of the bottle of concentrated wash solution to 500 mL, taking care also to transfer crystals completely, then mix until crystals are completely dissolved.

6.3. Preparation of the sample

The specimens should be human serum or plasma; the usual precautions in the collection of venipuncture samples should be observed.

For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

To obtain the serum, the blood should be collected in a venipuncture tube without additives or anti-coagulants; allow the blood to clot; centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of 5 days. If the specimens cannot be assayed within this time, the samples may be stored at -20°C for up to 30 days. Avoid repetitive freezing and thawing.

When assayed in duplicate, 0.100 mL of the specimen is required.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C).**
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C₀-C₅), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/Control	Blank
Sample /Control		50 µL	
Calibrator C ₀ -C ₅	50 µL		
Conjugate	100 µL	100 µL	
Incubate at room temperature (22-28°C) for 1 hours. Remove the contents from each well. Wash the wells 3 times with 300 µL of diluted Wash Solution. Important note: gently shake the plate for 5 seconds at each washing step to ensure proper cleaning. <u>After the last wash remove excess solution by tapping the inverted plate on an absorbent paper towel.</u>			
TMB Substrate	100 µL	100 µL	100 µL
Incubate at room temperature (22-28°C) for 15 minutes in the dark.			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank within 5 minutes.			

7. QUALITY CONTROL

Each laboratory should assay controls at levels of a low, normal, and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations. If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbance (E_m) for each point of the calibration curve (C₀-C₅) and of each sample.

8.2. Calibration Curve

Plot the mean value of absorbance (E_m) of the calibrators (C₀-C₅) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

8.3. Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in mIU/mL.

9. EXPECTED VALUES

Reference ranges are reported below:

	FSH (mIU/mL)
MALE	1 – 14
FEMALE:	
Follicular phase	3 – 12
Mid-cycle	8 – 22
Luteal phase	2 – 12
Menopausal	35 – 151

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. LIMITATIONS OF THE PROCEDURE

- **For diagnostic purposes FSH values should be used as an adjunct to other data available to the physician.** Procedural directions must be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results. FSH is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination of FSH alone is not sufficient to assess clinical status.
- FSH is suppressed by estrogen but in woman taking contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentration.
- Patients specimens with abnormally high FSH levels can cause a hook effect, that is, paradoxical low absorbance results. If this is suspected, dilute the specimen 1:100 with the Calibrator 0, re-assay (multiply the result by 100). However, values as high as 2000 mIU/mL have been found to absorb greater than the absorbance of the highest calibrator.
- Patients receiving preparations of mouse monoclonal antibodies for diagnosis of therapy may contain human anti-mouse antibodies (HAMA) and may show either falsely elevated or depressed values when assayed.

11. PERFORMANCE AND CHARACTERISTICS

11.1. Precision

11.1.1. Intra-Assay

Within-run precision was determined by replicate (20x) the measurement of three different control sera in one assay. The within-assay variability is ≤ 9.7%.

11.1.2. Inter-Assay

Between-run precision was determined by replicate (10x) the measurement of three different control sera in different lots of kits. The between-assay variability is ≤ 8.0%.

11.2. Correlation

Diametra FSH kit (Y) was compared to a commercially available FSH kit (X). 35 serum samples have been tested. The linear regression curve was:

$$Y = 0.91 \cdot X + 3.41$$

$$r^2 = 0.969$$

The new Diametra FSH kit (Y) was compared to the previous Diametra FSH kit (X): 60 serum samples have been tested. The linear regression curve was:

$$Y = 1.01 \cdot X - 0.44$$

$$r^2 = 0.953$$

11.3. Sensitivity

The minimal detectable concentration of FSH that can be distinguished from the Calibrator 0 is 0.17 mIU/mL.

11.4. Specificity

The cross-reactivity of the Diametra FSH ELISA method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Follicle Stimulating Hormone needed to produce the same OD.

Substance	Concentration	Cross Reactivity
FSH	---	100,0%
HCG	5000 mIU/mL	N.D.
LH	400 mIU/mL	N.D.
Prolactin	2000 ng/mL	N.D.
TSH	1000 mIU/L	N.D.

11.5. Accuracy

Three different samples were spiked with 3, 6, 12 and 24 mIU/mL of FSH; the recovery test showed an average value (± SD) of 97.83% ± 8.52%.

Three different samples were diluted 2, 4 and 8 times with the Calibrator 0; the dilution test showed an average value (± SD) of 105.54% ± 5.36%.

12. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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