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ELISA **E**NZYME **L**INKED **I**MMUNO**S**ORBENT **A**SSAY

Microwell Method

FSH

(Follicle Stimulating Hormone)

Cat. No. Z05302

P r o d u c t I n s e r t

CE IVD

Enzyme Linked Immunosorbent Assay for the **quantitative** determination of Follicle Stimulating Hormone in human serum or plasma.

Microwell Method - 96 wells

(12 x 8-well Antibody coated Strips)

Individual breakaway

GENERAL INFORMATION

❑ **Wavelength**

Measurement Filter: 450 nm

❑ **Incubation Time**

80 minutes at RT (60/20)

❑ **Enzyme Conjugate**

HRP (Horseradish Peroxidase)

❑ **Substrate**

TMB (3,3´,5,5´-Tetramethyl-benzidine)

❑ **Sample**

Serum or Plasma

❑ **Stability of Samples**

undiluted: 2 days at 2-8°C; for longer storage at - 20 °C

❑ **Calibration Range**

1 - 100 mIU/ml

❑ **Sensitivity**

1 mIU/ml

❑ **Shelf life and Stability of Kit Components**

Kit:	18 months from production date.
Kit Components:	see expiration date on the label

KIT COMPONENTS

Microwell plate	12x	**8 well strips** with breakaway microwells coated with anti-monoclonal FSH.
Calibrators	5	vials, A 1 ml, B-E 0.4 ml, **ready to use.** Aprox. 0, 5; 12; 40; and 100 mIU/ml, see labels
Enzyme Conjugate	1	vial of anti-monoclonal FSH HRP conjugate, 11 ml, **ready to use** .
Substrate Solution	1	vial of H_2O_2,TMB 0.25 g/l, 11 ml, **ready to use.**
Stop Solution	1	vial of sulphuric acid 0.15 mol/l, 11 ml, **ready to use**.

MATERIALS REQUIRED BUT NOT PROVIDED

- ❑ Deionized or distilled water
- ❑ Graduated cylinders and beakers
- ❑ Wash trays
- ❑ **Macropipettes** capable of delivering 5 µl to 1000 µl.
- ❑ **Multichannel Micropipette**
- ❑ **Stepper**
- ❑ **Microplate reader** capable of reading absorbance values at 450 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-690 nm.
- ❑ **Automatic microplate washer** capable of dispensing 200 µl.

Microplate reader and microplate washers are available from Dialab Company.

SUMMARY AND EXPLANATION

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 Iow. 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.

TEST PRINCIPLES

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.

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	1.2 – 19.3
Female:	
Follicular phase	3.9 – 8.8
Ovulatorial phase	4.5 – 22.5
Luteal phase	1.8 – 5.1
Post Menopausal	16.7 – 113.6
Pregnant female	0.0 – 11.6
Children, prepubertal	< 1.0

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

REAGENTS

Storage

- ❑ Store all reagents at 2° - 8°C. Do not freeze!

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`-Tetramethyl-benzidine)**. 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 DIALAB.
- ❑ 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 HANDLING

- ❑ Only **Serum or Plasma** specimens should be used in this procedure. The patients need not to be fasting, and no special preparations are necessary.
- ❑ 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.
- ❑ Store specimens at **2°- 8°C** for up to a maximum of **2 days**. For longer storage, specimens should be frozen. Avoid repeated freezing and thawing of samples.
- ❑ For sample with concentration over 100 mIU/ml dilute the sample 1/1 with Calibrator A.

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 200 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 100 mIU/ml 1:1 with Calibrator A.

Test Procedure

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

	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										

Calibrator	Conc. mIU/ml
SA	0
SB	5
SC	12
SD	40
SE	100

Step 4 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.

Step 5 Pipette **50 µl** of **Calibrators and Patient Samples** into the wells.

Step 6 Add 100 µl of diluted Enzyme Conjugate to the wells except for Blank well and incubate **60 minutes** at room temperature.

Step 7 Discard the contents of the microwells and wash the wells with **200 µl** distilled water. Repeat 2 x the washing procedure by draining the water completely.

Step 8 Pipette **100 µl of Substrate Solution** into each microwell in the same order and timing as for the Enzyme Conjugate, Blank well included.

Step 9 Incubate **20 minutes** at room temperature in the dark.

Step 10 Add **100 µl of Stop Solution** into each microwell using the same order and timing as for the addition of the Substrate Solution.

Step 11 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 calibrator from the mean absorbance values of calibrators and samples.
3. Draw the standard curve on log-log graph paper by plotting absorbance values of standard against appropriate FSH concentration.
4. Read off the FSH concentrations of the control and samples.

LIMITATIONS OF THE PROCEDURE

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.

Q.C. PARAMETERS

Maximum Absorbance (calibrator E) = OD (100 mIU/ml) = min. 50% of the value stated in quality certificate.

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	< 1 %
HCG	< 1 %
PRL	< 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	14	14	14
Mean FSH (mIU/ml)	6,7	11,8	49,7
Coef. of Variation (%)	8,6	6,5	4,2

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	12	12	12
Mean FSH (mIU/ml)	6,45	12,6	49
Coef. of Variation (%)	11,6	8,6	8,1

7.4 Recovery

Expected conc.	Observed conc.	Recovery
35,5	37,6	105,6
65,7	67,2	102,3
75,1	72,0	95,9
81,3	82,7	101,7

7.5 Linearity

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

Dilution	Exp. Conc	Obs. Conc	Recovery
		46	
Dil. 1 / 2	23	25,1	109,1
Dil. 1 / 4	11,5	11,2	97,4
Dil. 1 / 8	5,75	5,83	101,4

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ELISA **E**nzyme **L**inked **I**mmuno**s**orbent **A**ssay

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