AB Diagnostic Systems

REF **DK.042.01.9** Σ **96**

CE

IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia FSH
Enzyme immunoassay for the quantitative determination of follicle stimulating hormone (FSH) concentration in human serum

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 (breakable wells) assays including controls; partial use of the kit is possible; can be used for manual protocol.

I. INTENDED USE

The abia FSH kit is intended for the quantitative determination of Follicle Stimulating Hormone (FSH) concentration in human serum by a microplate immunoenzymometric assay.

This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. All the reagents are for professional *in vitro* diagnostic use only.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture.

II. INTRODUCTION

FSH is a glycoprotein secreted by the basophile cells of the anterior pituitary. Gonadotropin-releasing hormone (GnRH), produced in the hypothalamus, controls the release of FSH from the anterior pituitary. Like other glycoproteins, such as LH, TSH, and HCG, FSH consists of subunits designated as alpha and beta. Hormones of this type have alpha subunits that are very similar structurally, therefore the biological and immunological properties of each are dependent on the unique beta subunit. In the female, FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells; follicular steroidogenesis is promoted and LH production is stimulated. The LH produced then binds to the theca cells and stimulates steroidogenesis. Increased intraovarian estradiol production occurs as follicular maturation advances, thereupon stimulating increased FSH receptor activity and FSH follicular binding. FSH, LH, and estradiol are therefore intimately related in supporting ovarian recruitment and maturation in women.

FSH levels are elevated after menopause, castration, and in premature ovarian failure. The levels of FSH may be normalized through the administration of estrogens, which demonstrate a negative feedback mechanism. Abnormal relationships between FSH and LH, between FSH and estrogen have been linked to anorexia nervosa and polycystic ovarian disease. Tumors of the testes generally depress serum FSH concentrations, but levels of LH are elevated, as determined by radioimmunoassay. It has been postulated that the apparent LH increase may be caused by cross reactivity with hCG-like substances secreted by tumors of the testes. High levels of FSH in men may be found in primary testicular failure and Klinefelter syndrome. Elevated concentrations are also present in cases of starvation, renal failure, hyperthyroidism and cirrhosis.

III. PRINCIPLE OF THE TEST

The abia FSH is a one-step immunoassay, based on principle of “sandwich” method. The assay system utilizes a high affinity and specificity monoclonal antibody (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact FSH molecule. The test sample is allowed to react simultaneously with the two antibodies, resulting in the FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed with washing solution to remove unbound labeled antibodies. A solution of TMB-Substrate is added and incubated, resulting in the development of a blue color. The color development is stopped with the addition of Stopping Reagent, changing the color to yellow. The concentration of FSH is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

IV. CONTENT OF THE KIT abia FSH

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Anti-FSH-coated microtiter wells	Polystyrene stripped 96-well plate (breakable wells) coated with monoclonal antibodies to FSH. Once opened, microtiter wells should be stored at 2-8 °C until expiration date of the kit.	1 plate
Conjugate	Monoclonal anti-FSH antibodies conjugated to horseradish peroxidase. Transparent or opalescent pink color liquid. Preserving agent: 0.1% ProClin 300, 0.004% gentamycin sulfate. Once opened, Conjugate should be used within two months. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.0 ml
Calibrator 0 Calibrator 1 Calibrator 2 Calibrator 3 Calibrator 4 Calibrator 5	Six vials containing FSH in protein-based buffer. Calibrators were calibrated using a WHO 3nd IRP 94/632. The FSH concentration levels in Calibrators are provided on the labels of vials and in the Certificate of Analysis on a lot-specific basis. Transparent or slightly opalescent liquids, pale yellow. Preserving agent: 0.1% ProClin 300, 0.004% gentamycin sulfate, 0.1% phenol. Once opened, Calibrators should be used within two months. Store at 2-8 °C until expiration date in a tightly sealed vial.	5 vials 0.5 ml Calibrator 0 – 2.0 ml
Control Serum	Control, human serum based. Transparent or slightly opalescent liquid, pale yellow. The FSH concentration level in Serum is provided on the vial label and in the Certificate of Analysis on a lot-specific basis. Preserving agent: 0.1% ProClin 300, 0.004% gentamycin sulfate, 0.1% phenol. Once opened, Control Serum should be used within two months. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 0.5 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (pH 6.9-7.7). Transparent or slightly opalescent liquid, colorless, or pale yellow, sediment may form that dissolves completely at 35-39 °C and shaking. Once opened, Washing Solution should be stored at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml
TMB-Substrate	Tetramethylbenzidine (0.03%) in citric acid buffer, containing H_2O_2 (0.01%). Transparent colorless liquid. Once opened, TMB-Substrate should be used within two months. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 14.0 ml
Stopping Reagent	0.2M sulfuric acid solution. Transparent colorless liquid. Once opened, Stopping Reagent should be stored at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml

Additionally the following may be included in the delivery set:

- a lid for polystyrene 96-well plates or a protective film for EIA plates;
- disposable tips;
- a plastic dish for liquid reagents;
- polyethylene bag with a Zip-Lock.

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the lab should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. In case of label loss or labels/vials damage, vials should be disposed and **kit cannot be used.**
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugates.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or TMB-Substrate.
- Use a new distribution tip for each sample.
- Do not reuse protective films for EIA plates.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and other solutions.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use distilled or deionized water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.
- Once the assay has been started, all subsequent steps should be performed without interruption.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Human origin material used in the preparation of Control Serum and Calibrators has been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).
- Certain reagents contain biological material of animal origin.

- Because no known test method can offer complete assurance that infections agents are absent, handle reagents and patients samples as if capable of transmitting infections disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples.
- Avoid any contact of the TMB-Substrate and the Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- All materials contacted with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations.

Warning!

Conjugate, Calibrators 0-5, Control Serum contain ProClin 300.

H317: May cause an allergic skin reaction.
P261: Avoid breathing vapors.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352: IF ON SKIN: Wash with plenty of water.
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention

Danger!

Stopping Reagent contains 0.2M sulfuric acid.

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT:

- Deionised or distilled water.
- Automatic or semiautomatic, adjustable or preset single-channel and multi-channel pipettes with a changeable volume for a set of liquids.
- Disposable pipette tips.
- Automatic microplate washer.
- Microplate reader equipped with 450 and 405-415 nm filter.
- Open type automated analyzer with 450 and 405-415 nm filter (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Blood samples should be collected according to the current practices Serum Serum only may be used. Separate serum as soon as possible to avoid any hemolysis. Extensive hemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

Store/transport the samples in accordance with the current regulatory documentation. If samples are to be stored/transported for a longer period of time, they must be frozen at or below -20 °C. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed bacterial growing, hemolysis, hyperlipidemia and which were preserved by sodium azide, thimerosal must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **Anti-FSH-coated microtiter wells.** Each 12-strips plate (breakable wells) is wrapped in a sealed foil-lined bag. Open the bag and remove the plate. Select the number of strips/wells required for the assay. Place the unused strips/wells back into the foil-lined bag; reseal the foil-lined bag in a Zip-Lock plastic bag. Do not remove desiccant.
- **Calibrators 0-5;**
- **Control Serum;**
- **Conjugate;**
- **TMB-Substrate;**
- **Stopping Reagent.**

2. Reagents to prepare:

- **Working Washing Solution**. Thoroughly shake Washing Solution concentrate. To make Working Washing Solution take required amount of concentrate and mix with distilled or deionized water (1:24 ratio) in a separate vial. The prepared Working Thoroughly mix the solution. The prepared Working Washing Solution is stable for 14 days at room temperature or 28 days at 2-8 °C in clean tightly sealed container.

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature for 30 min.

1. To the wells add 25 µl of Calibrators and Control Serum in duplicate. Leave two wells for OD control of TMB-Substrate.

2. To the rest of the wells, add 25 µl of samples in duplicate. Pipetting of samples should not extend beyond ten (10) minutes

3. Pipette 100 µl Conjugate to all wells except for the wells for OD control of TMB-Substrate.

4. Swirl the microplate gently for 30 seconds after adding of samples and Conjugate to mix, cover the strips with a lid or a protective film and incubate for 90 minutes at room temperature (here 20-25 °C).

5. Aspirate the contents of the wells into the container with disinfecting solution. Wash the wells 5 times with 300 µl of Working Washing Solution per well and remove Working Washing Solution using a washer into the container with disinfecting solution. Tap the plate firmly against absorbance paper to ensure that it is dry – the residual volume must be lower than 10 µl (the use of a washer is recommended). Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.

6. Pipette 100 µl of TMB-Substrate into each well.

7. Incubate for 15-20 minutes at room temperature in a dark place.

8. Pipette 150 µl of Stopping Reagent into each well. Gently mix for 5-10 seconds.

9. Read the plate on microplate reader at 450 nm. In case of overflow absorbance values, read at 405-415 nm. Reading must be completed within 20 minutes after addition of the Stopping Reagent.

Scheme of the assay is represented in Annex.

Spectrophotometric verification of reagent pipetting

The presence of Conjugate + sample in the well can be verified by automatic reading at 540 (550) nm. Each well containing sample and Conjugate must have an OD higher than 0.500.

10. Automated analyzers

Validated test protocols and dilution tables of reagent working solutions for different EIA-analyzers can be obtained from the manufacturer upon request (see section XIV). For the instrumentation without established validated protocol follow the section “TEST PROCEDURE” and ensure all requirements described in the section “PRECAUTIONS” are fulfilled. All protocols for automated analyzers must be fully validated before use.

When preparing working reagent solutions for automated EIA procedure, it is necessary to consider “dead” volume of vials and containers used for loading working solutions in the EIA analyzer.

XI. CALCULATION OF RESULTS

1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the “blank” from the mean absorbance values of the Calibrators, Control Serum and serum samples.
4. Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
5. In case of overflow absorbance values at 450 nm, read the results at 405-415 nm.
6. If a sample reads more than value of Calibrator 5 then dilute it with Calibrator 0. The result obtained should be multiplied by the dilution factor.

Typical tabulated data:

Calibrator	OD1	OD2	Mean OD-blank	Value (mIU/ml)
0	0.044	0.040	0.042	0
1	0.257	0.261	0.259	5
2	0.45	0.47	0.46	10
3	1.012	1.015	1.014	25
4	1.780	1.82	1.80	50
5	2.75	2.8	2.78	100
Unknown	0.42	0.4	0.41	**8.4**

This data is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Test Validation

For the test to be valid, the following criteria must be met. If these criteria are not met, the test should be considered invalid and should be repeated.

1. **Blank OD:** The absorbance value should not be more than 0.1 at 450 nm.
2. The absorbance (OD) of **Calibrator 5** should not be less than 1.3.
3. Calculated Value of **Control Serum** should be within established range.

XII. PERFORMANCE CHARACTERISTICS OF abia FSH

1. Assay Dynamic Range

The range of the assay is between 0-100 mIU/ml.

2. Analytical sensitivity

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator 0 (based on 12 replicate analyses) plus 2 SD.

Therefore, the sensitivity of the abia FSH kit is **0.3 mIU/ml.**

3. Specificity (cross reactivity)

The following substances were tested for cross reactivity of the assay:

Cross-reagent	Cross reactivity, %
FSH	100
hCG	0.001
TSH	0.0000
LH	0.019

4. Precision

Intra-assay precision

The within assay variability is shown below:

Sample	n	Mean, mIU/ml	SD	CV, %
1	9	10.1	0.450	4.5

Inter-Assay precision

The between assay variability is shown below:

Sample	Mean, mIU/ml	SD	CV, %
1	9.7	0.442	4.6

5. Recovery

Spiked samples were prepared by adding defined amounts of FSH to patient serum sample. The results are tabulated below:

Added conc., mIU/ml	Measured Conc. mIU/ml	Expected Conc. mIU/ml	Recovery, %
-	9.9	9.9	
10	9.7	9.9	97

6. Linearity

Patient serum sample were diluted with Calibrator 0. The results are tabulated below:

Sample	Measured Conc. mIU/ml	Expected Conc. mIU/ml	Recovery, %
1 (90 mIU/ml) : 2	41.2	45	92
1 (45 mIU/ml) : 2	21.2	22.5	94
1 (23 mIU/ml) : 2	11.5	11.5	100

7. Expected normal Value

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Group		Value, mIU/ml
Women	Follicular phase	< 10
	Midcycle	5-16
	Luteal phase	< 10
	Postmenopausal	25-150
Men		1.4-14

8. Accuracy

The abia FSH kit was compared with a Chemiluminescent microparticle immunoassay as a reference test. The total number of specimens was 937. The values ranged from 0.1 to 141 mIU/ml. The least square regression equation and the correlation coefficient were computed for abia FSH in comparison with the reference method. The least square regression analysis was **y = 1.06(x) - 0.53** with correlation coefficient 0.98.

XIII. LIMITS OF THE TEST

1. All the reagents within the kit are calibrated for the direct determination of FSH in human serum. The kit is not calibrated for the determination of FSH in saliva, plasma or other specimens of human or animal origin.
2. Any improper handing of samples or modification of this test might influence the results.
3. Only Calibrator 0 may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
4. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient.
5. Some individuals may have heterophilic antibodies to mouse or other animal proteins that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.
6. No hook effect was observed in this test.
7. Not intended for newborn screening.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

Expiry date is indicated on the packaging.

Keep in dark dry place at 2-8 °C. Freezing is prohibited.

Transportation should be done at 2-8 °C. Transportation at 9-20 °C is allowed not more than during ten (10) days.

AB Diagnostic Systems GmbH
Sportfliegerstraße 4, Berlin, 12487, Germany
Tel. +49 30 208987160, Fax: +49 30 208987199
E-Mail: info@ab-ds.de, www.ab-ds.de

XV. REFERENCES

1. Harris, G.W. and Naftolinf., The Hypothalamus and Control of Ovulation, Brit. Med. Bullet., 26, 1-9 (1970).

2. Knobil, E., The Neuroendocrine Control of the Menstrual Cycle, Rec. Prog. Horm Res., 36, 52-88 (1980).

3. Jeffcoate, S.L., Clinics in Endocrinol. Metab., 4, 521-543 (1975).

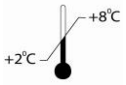
4. Pierce, J.G. and Parsons, T.F., Glycoprotein hormones: Structure and Function, Annual Rev. Biochem., 50, 465-495 (1981).

5. Ross, G.T., VandeWeile, R.L., and Frantz, A.G. Chapter 7 in “The Ovaries and the Breasts” in “Textbook of Endocrinology” (R. H. Williams, Ed.), W.B. Saunders Co. (1981).

6. Engvall, E., Methods in Endocrinology, Volume 70, Van Vunakis, H. and Langone, J.J. (eds.), Academic Press, New York, 419-492 (1980).

7. Uotila, M. Ruoslahti, E. and Engvall, E., J. Immunol. Methods, 42, 11-15 (1981).

XVI. EXPLANATION OF SYMBOLS

CE	CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)		Storage temperature limitation
	Manufacturer		Consult Instruction for use
	Date of manufacture CCYY-MM	IVD	For in vitro diagnostic use
	Expiry date CCYY-MM-DD		Sufficient for
LOT	Batch code		Symbol “exclamation mark”
REF	Catalog number	**Warning!**	Signal word
	Fragile, handle with care		Symbol “corrosion”
	Keep away from sunlight	**Danger!**	Signal word
	Keep dry		Top

Annex

Scheme of the assay

1	**Add**	25 µl of Calibrators, Control Serum in duplicates; 25 µl of samples in duplicates; two wells for OD control of TMB-Substrate
2	**Add**	100 µl of Conjugate into all wells, except for the wells for OD control of TMB-Substrate
3	**Mix**	30 seconds
4	**Incubate**	90 min, at 20-25 °C
5	**Wash the plate**	**Working Washing Solution, 300 µl, 5 times**
6	**Add**	100 µl of TMB-Substrate into all wells
7	**Incubate**	15-20 min, at room temperature in a dark place
8	**Add**	150 µl of Stopping Reagent into all wells
9	**Mix**	5-10 seconds
10	**Read the optical density**	450 nm, 405-415 nm