AB Diagnostic Systems

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CE

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

INSTRUCTIONS FOR USE
abia LH
Enzyme immunoassay for the quantitative determination of luteinizing hormone (LH) 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 LH kit is intended for the quantitative determination of Luteinizing Hormone (LH) 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

Luteinizing hormone (LH) is produced in both men and women from the anterior pituitary gland in response to luteinizing hormone-releasing hormone (LH-RH or Gn-RH), which is released by the hypothalamus (1-3). LH is a glycoprotein with a molecular weight of approximately 30.000 daltons. It is composed of two non covalently associated dissimilar amino acid chains, alpha and beta. The alpha chain is similar to that found in human thyroid-stimulating hormone (TSH), follicle stimulating hormone (FSH), and human chorionic gonadotropin (hCG). The difference between these hormones lie in the amino acid composition of their beta subunits, which account for their immunological differentiation. The basal secretion of LH in men is episodic and has the primary function of stimulating the interstitial cells (Leydig cells) to produce testosterone. The variation in LH concentrations in women is subject to the complex ovulatory cycle of healthy menstruating women, and depends upon a sequence of hormonal events along the gonado-hypothalamic-pituitary axis. The decrease in progesterone and estradiol levels from the preceeding ovulation initiates each menstrual cycle. As a result of the decrease in hormone levels, the hypothalamus increases the secretion of gonadotropin-releasing factors (GnRF), which in turn stimulates the pituitary to increase FSH production and secretion. The rising FSH levels stimulate several follicles during the follicular phase, one of these will mature to contain the egg. As the follicle develops, estradiol is secreted, slowly at first, but by day 12 or 13 of a normal cycle increasing rapidly. LH is released as a result of this rapid estradiol rise because of direct stimulation of the pituitary and increasing GnRF and FSH levels. These events constitute the pre-ovulatory phase. Ovulation occurs approximately 12 to 18 hours after the LH reaches a maximum level. After the egg is released, corpus luteum is formed which secretes progesterone and estrogen – two feedback regulators of LH. The luteal phase rapidly follows this ovulatory phase, and is characterized by high progesterone levels, a second estradiol increase, and low LH and FSH levels. Low LH and FSH levels are the result of the negative feedback effects of estradiol and progesterone on the hypotalamic-pituitary axis.

After conception, the developing embryo produces hCG, which causes the corpus luteum to continue producing progesterone and estradiol. The corpus luteum regresses if pregnancy does not occur, and the corresponding drop in progesterone and estradiol levels results in menstruation.

The hypothalamus initiates the menstrual cycle again as a result of these low hormone levels. Patients suffering from hypogonadism show increased concentrations of serum LH. A decrease in steroid hormone production in females is a result of immature ovaries, primary ovarian failure, polycystic ovary disease, or menopause; in these cases, LH secretion is not regulated. In the differential diagnosis of hypothalamic, pituitary, or gonadal dysfunction, assays of LH concentration are routinely performed in conjunction with FSH assays since their roles are closely interrelated. Furthermore, the hormone levels are used to determine menopause, pinpoint ovulation, and monitor endocrine therapy.

III. PRINCIPLE OF THE TEST

The abia LH 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 LH molecule. The test sample is allowed to react simultaneously with the two antibodies, resulting in the LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed with washing buffer 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 LH is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

IV. CONTENT OF THE KIT abia LH

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Anti-LH-coated microtiter wells	Polystyrene stripped 96-well (break apart) plate coated with monoclonal anti-LH antibodies. Once opened, microtiter wells should be stored at 2-8 °C during shelf-life of the kit.	1 plate
Conjugate	Monoclonal anti-LH 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 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 LH in protein-based buffer. The calibrators were calibrated using a WHO 2nd IRP 80/552. The LH 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 in a tightly sealed vials.	5 vials 0.5 ml. Calibrator 0 2.0 ml
Control Serum	Control, human serum based. The LH concentration level in Serum is provided on the vial label and in the Certificate of Analysis on a lot-specific basis. Transparent or opalescent liquid, pale yellow. 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 in a tightly sealed vial.	1 vial 0.5 ml
Washing Solution (concentrated 25-fold)	Transparent or slightly opalescent liquid, colorless, or pale yellow. Once opened, Washing Solution should be stored at 2-8 °C until the expiry date of the kit.	1 vial 50.0 ml

TMB-Substrate	Tetramethylbenzidine in citric acid buffer, containing H_2O_2. Transparent colorless liquid. Once opened, TMB-Substrate should be used within two months. Store at 2-8 °C 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 the expiry date of the kit.	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 18-25 °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 substrate solutions.
- Use a new distribution tip for each sample.
- Do not reuse protective films for EIA plates.
- Do not let the wells dry once the assay has been started.
- 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 color development solution.
- 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 vapours.
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:

- Distilled or deionized 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 nm filter.
- Open type automated analyzer with 450 nm filter (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Collection of blood samples should be implemented according to the current practices. 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. The blood should be collected in plain redtop venipuncture tube without additives and gel barrier.

Samples can be stored at 2-8 °C not more than for 72 hours; they may be deep-frozen at -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 must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **Anti-LH-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. 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 for30 min.

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

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. Add 100 µl of 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 plate 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 absorbent 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. Stop the reaction by adding 150 µl of Stopping Reagent to the wells, shake the strips for 5-10 seconds and read the results. The time between stopping the reaction and measuring OD should not exceed 20 min.

9. Read the absorbance on the microplate reader at 450 nm.

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 unknown duplicate.
2. 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.
3. Calculate the mean absorbance values for each specimen.
4. If a sample reads more than 100 mIU/ml 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	0.045	0.041	0	0
1	0.256	0.259	0.214	5
2	0.448	0.472	0.417	10
3	1.009	1.027	0.975	25
4	1.786	1.837	1.7685	50
5	2.763	2.939	2.808	100
Unknown	0.364	0.399	0.339	8.34

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 be more than 0.1 at 450 nm.
2. The absorbance (OD) of **Calibrator 5** (100 mIU/ml) should be less than 1.3.
3. Calculated Value of **Control Serum** should be within established range.

XII. PERFORMANCE CHARACTERISTICS OF abia LH

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 LH kit is **0.3 mIU/ml.**

3. Specificity (cross reactivity)

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

Substance	Cross reactivity,%
LH	100
hCG	0
TSH	0
FSH	0

4. Precision

Intra-assay precision

The within assay variability is shown below:

Sample	Mean, mIU/ml	SD	CV,%
1	11.2	0.5051	4.5

Inter-assay precision

The between assay variability is shown below:

Sample	Mean, mIU/ml	SD	CV,%
1	11.3	0.3697	3.3

5. Recovery

Spiked samples were prepared by adding defined amounts of LH to patient serum sample. The results (in µIU/ml) are tabulated below:

Added Concentration, mIU/ml	Measured Conc., mIU/ml	Expected Conc. mIU/ml	Recovery, %
-	**11.4**	**11.4**	**-**
9.5	**10.0**	**10.5**	**96**

6. Linearity

Patient serum sample was diluted with calibrator 0. The results (in µIU/ml) are tabulated below:

Sample	Measured Conc. mIU/ml	Expected Conc. mIU/ml	Recovery, %
1(91.0 mIU/ml) : 2	**43.2**	**45.5**	**95**
1 (45.5 mIU/ml) : 2	**21.0**	**22.75**	**92**
1 (22.5 mIU/ml) : 2	**11.0**	**11.25**	**98**

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	0.9 – 15.0
	Midcycle	15.0 – 90.0
	Luteal phase	0.8 – 15.0
	Postmenopausal	10.0 – 65.0
Men		1.2 – 9.0

8. Accuracy

The abia LH test system was compared with a Chemiluminescent microparticle immunoassay as a reference test. The total number of specimens was 803. The values ranged from 0.02 to 83.82 mIU/ml. The least square regression equation and the correlation coefficient were computed for abia LH in comparison with the reference method. The least square regression analysis was **y=0.021+1.003(x)** with correlation coefficient 0.95.

XIII. LIMITS OF THE TEST

1. All the reagents within the kit are calibrated for the direct determination of LH in human serum. The kit is not calibrated for the determination of LH in saliva, plasma or other specimens of human or animal origin.
2. Any improper handling 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. LH is suppressed by estrogen but in women taking oral contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentration.
6. 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.
7. No hook effect was observed in this test.
8. 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.

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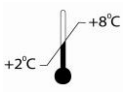
XV. REFERENCES

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 (blank)
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