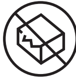
abia LH



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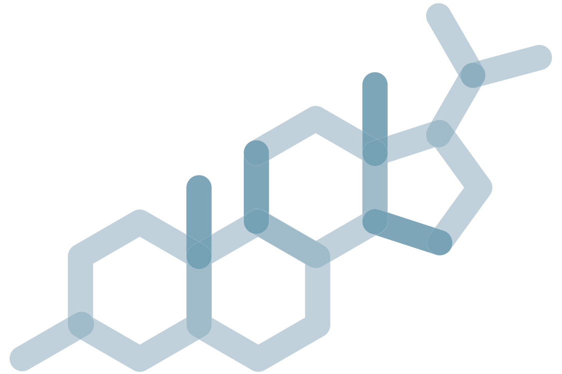

abia

Intended use

abia LH is an enzyme immunoassay for the quantitative determination of luteinizing hormone (LH) concentration in human serum.

For professional use only.

Clinical value

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. 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), while the β-subunit is unique. 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. 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.

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.

Increased concentrations of LH are also present during renal failure, cirrhosis, hyperthyroidism, and severe starvation. A lack of secretion by the anterior pituitary may cause lower LH levels. Low levels may result in infertility in both males and females. Low levels of LH may also be due to the decreased secretion of GnRH by the hypothalamus, although the same effect may be seen by a failure of the anterior pituitary to respond to GnRH stimulation. Low LH values may therefore indicate some dysfunction of the pituitary or hypothalamus, but the actual source of the problem must be confirmed by other tests.

Principle of the test

abia LH is a one-step immunoassay, based on the principle of the “sandwich” method.

The assay system utilizes high affinity and specificity monoclonal antibodies (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.

The unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue colour which is converted to yellow after the reaction has been stopped with sulphuric acid.

The colour intensity is directly proportional to the concentration of LH in the specimen and can be read at 450 nm.

Kit contents

	S	
LH Ab coated plate	1	polystyrene plate 12 × breakable 8-well strips coated with monoclonal anti-LH antibodies
Conjugate	1 × 12 ml	ready to use; HRP-labeled monoclonal anti-LH antibodies; transparent or slightly opalescent pink liquid
Calibrator 0	1 × 2.0 ml	protein based buffer not containing LH; pale yellow liquid
Calibrator 1	1 × 0.5 ml	protein based buffer containing LH in concentration approx. 5 mIU/ml; pale yellow liquid
Calibrator 2	1 × 0.5 ml	protein based buffer containing LH in concentration approx. 10 mIU/ml; pale yellow liquid
Calibrator 3	1 × 0.5 ml	protein based buffer containing LH in concentration approx. 25 mIU/ml; pale yellow liquid
Calibrator 4	1 × 0.5 ml	protein based buffer containing LH in concentration approx. 50 mIU/ml; pale yellow liquid
Calibrator 5	1 × 0.5 ml	protein based buffer containing LH in concentration approx. 100 mIU/ml; pale yellow liquid
Control serum	1 × 0.5 ml	protein based control containing LH; colourless or pale yellow
Washing solution (concentrated 25-fold)	1 × 50 ml	phosphate saline buffer; colourless or pale yellow liquid
TMB/substrate solution	1 × 12 ml	ready to use; citric acid buffer containing TMB and H_2O_2; colourless liquid
Stopping reagent 0.2M H_2SO_4	1 × 25 ml	ready to use; 0.20 mol/l sulphuric acid solution; colourless liquid
Protective film	1	
Plastic dish	2	
Plastic zip-lock bag	1	

The calibrators were calibrated using a WHO 2nd IRP 80/552. Exact concentration levels for calibrators and control serum are given on the labels on a lot specific basis.

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package.
Once opened, the components should be used within 1 month. Concentration of preserving agents: <=0.1 %.

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- automatic microplate washer
- microplate reader equipped with 450 nm filter

Safety notes

- as no known test method can offer complete assurance that infections agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum as soon as possible to avoid any haemolysis
- do not use sera preserved with sodium azide, thiomersal
- do not use contaminated, hyperlipaemic and hyperhaemolysed specimens
- the samples with hyperproteinaemia and hyperbilirubinaemia were not specially tested
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- samples can be stored at 2–8 °C within 72 hours or deep-frozen at -20 °C
- no more than one freeze/thaw cycle is allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (20–25 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section “Washing procedure”
- for the description of test procedure with the automated analyzers see section “Automated analyzers”

Washing procedure

Please contact your representative for protocols for recommended washers and procedures.
In general the following protocol is recommended:

- flow-through washing with a volume not less than 300 μl per well is used
- repeat 5 times
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 μl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion **Stability:** 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0

Test procedure

abia LH for the quantitative determination of luteinizing hormone (LH) concentration in human serum

1	Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in plastic zip-lock bag. Do not remove desiccant.
2	Analyse each calibrator, control serum, and sample duplicate. Reserve one or two wells for TMB/substrate solution control (blank). Add 25 µl of calibrators 0 - 5 into appropriate wells. Add 25 µl of control serum into appropriate wells. Add 25 µl of samples to be tested in rest of the wells. The total time should not exceed 10 min.
3	Add 100 µl conjugate into each well except blank. Mix the contents of the wells for 30 seconds by careful tapping on the edge of the plate, then cover the plate with protective film.
4	Incubate for 90 minutes at room temperature 20–25 °C.
5	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 300 µl of working washing solution into each well and aspirate. Perform this procedure 5 times. Use double aspiration in the final step where possible.
6	Add 100 µl of TMB/substrate solution to all the wells. Keep the plates in a dark place for 15–20 minutes at 20–25 °C.
7	Add 150 µl of stopping reagent into each well. Mix gently for 5–10 sec.
8	Read the optical density at 450 nm using a plate reader within 20 minutes after stopping reaction.

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative.
For the instrumentation without established validated protocol follow section "Test procedure" and ensure all requirements described in section "Precautions" are followed.
All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of blank value should be not more than 0.100 at 450 nm.
The absorbance (OD) of calibrator 5 (approx 100 mIU/ml) should be greater than 1.300.
Calculated value of control serum should be within established range.

Calculation procedure

1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each sample duplicate.
3. Subtract the mean absorbance value of the "blank" from the mean absorbance values of the calibrators, control 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.
5. Read the values of the samples directly off the calibration curve.
   If immunoassay software is being used, a 4-parameter curve is recommended.

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.

Example	OD 1	OD 2	Mean OD - blank (here 0.043)	Value, mIU/ml
Calibrator 0	0.045	0.041	0.000	0.00
Calibrator 1	0.256	0.259	0.214	5.00
Calibrator 2	0.448	0.472	0.417	10.00
Calibrator 3	1.009	1.027	0.975	25.00
Calibrator 4	1.786	1.837	1.769	50.00
Calibrator 5	2.763	2.939	2.808	100.00
Sample	0.364	0.399	0.339	8.34

This data is for illustration only and should **not be used** to calculate samples. Each user should obtain his or her own data and standard curve.

Performance characteristics

Analytical sensitivity

The analytical sensitivity (limit of detection) was calculated by determining the variability of the calibrator 0 based on 12 analysis runs additional 2 x SD. Limit of detection defined at 0.30 mIU/ml.

Specifity	Concentration, mIU/ml	Cross reactivty, %
Chorionic gonadotropin (hCG)	50 000	< 0.0001
Follicle-stimulating hormone (FSH)	50 000	< 0.0001
Thyroid-stimulating hormone (TSH)	1	< 0.0001

Precision	Mean, mIU/ml	SD	CV, %
Intra-assay, sample 1	11.20	0.505	4.50
Inter-assay, sample 1	11.30	0.370	3.30

Accuracy

The assay 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 $y = 1.003(x) + 0.021$ with correlation coefficient 0.95.

Expected normal value	Range, mIU/ml	
Female		
Follicular phase	0.90	15.00
Ovulatory phase	15.00	90.00
Luteal phase	0.80	15.00
Postmenopause	10.00	65.00
Male	1.20	9.00

Normal value ranges may vary slightly among different laboratories. It is strongly recommended that each laboratory should determine its own range of expected normal values.

Limitations of test

- the assay was validated only for the determination of LH in human serum
- only calibrator 0 may be used to dilute any high serum samples. The use of any other reagent may lead to false results
- the results obtained with this assay 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
- 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
- the assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results
- no hook effect was observed in this test
- not intended for newborn screening

References

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Key to symbols used

Symbol	Meaning
	Manufacturer
	For in vitro diagnostic use
	Catalogue number
	Batch code
	Expiry date
	Storage temperature limitation
	Do not use if package is damaged
	Do not reuse
	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
	Changes highlighted

Hazard and precautionary statements for certain kit components

Warning

Stopping reagent

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Warning

Conjugate, calibrators 0 - 5, control serum

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

Attention!
For complete precautionary statements and detailed information see safety data sheets (SDS).

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