Instruction for use for SKU LTHSI202K

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF LH IN HUMAN BLOOD SERUM OR PLASMA

1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of LH in blood serum or plasma.

This kit is designed for measurement of LH in blood serum or plasma. For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 42 unknown samples in duplicates.

2. SUMMARY AND EXPLANATION

Luteinizing hormone (LH) is produced in both men and women by the anterior pituitary gland in response to luteinizing hormone-releasing hormone (LH-RH or Gn-RH), which is released by the hypothalamus. LH, also called interstitial cell-stimulating hormone (ICSH) in men, is a glycoprotein with a molecular weight of approximately 30,000 daltons. It is composed of two noncovalently associated amino acid chains: alpha and beta.

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 on sequence of hormonal events along the gonado-hypothalamus-pituitary axis. During the cycle, LH level is low except for the middle of the cycle when its concentration may increase up to 5–10 fold. LH peak is preceeded by a peak of Estradiol which occurs approximately 12 hours earlier. Ovulation occurs 12-120 hrs after LH peak. When the ovum is released, the corpus luteum is formed which secretes progesterone and estradiol, these latter exerting negative feedback effects on LH and FSH levels through hypothalamo-pituitary axis.

LH concentration in blood is subject to circadian rhythms; therefore blood sample for LH assay should always be taken at the same time of the day. Circadian variations of LH level are more pronounced in women depending of the stage of menstrual cycle: they become less frequent at the end of luteinic phase and less pronounced – at the end of follicular stage. Increased LH levels are found in primary dysfunction of gonadal glands, in amenorrhea caused by ovarian insufficiency, in Stein-Leventhal syndrome, after menopause. Increased concentrations of LH are also present during renal failure, cirrhosis, hyperthyroidism, and severe starvation.

Decreased LH concentrations are seen in dysfunction of hypophysis or hypothalamus, in galactorrhea-amenorrhea syndrome, in isolated decrease of gonadotropins, in isolated LH decrease; in neurotic anorexia, in patients with retardation of growth and sexual development, after intake of digoxin, phenotiazine, progesterone, estrogens.

In the differential diagnosis of hypothalamic, pituitary, or gonadal dysfunction, assays of LH concentration are routinely performed in conjugation with FSH assays since their roles are closely interrelated. Furthermore, the hormone levels are used to determine menopause, pinpoint ovulation, and monitor endocrine therapy.

3. PRINCIPLE OF THE TEST

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to β chain human LH-antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Second antibodies – murine monocnoclonal to α chain human LH/FSH/HCG, labelled with peroxidase enzyme, are then added into the microwells. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

4. WARNINGS AND PRECAUTIONS

4.1. To be used by Laboratory Professionals only.

4.2. This kit is intended for research use only.

4.3. INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

4.4. Avoid contact with stop solution containing 5,0 % H_2SO_4. It may cause skin irritation and burns.

4.5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.

4.6. Do not use the kit beyond the expiration date.

4.7. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.

4.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

4.9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

4.10. Do not mix reagents from different lots.

4.11. Replace caps on reagents immediately. Do not swap caps.

4.12. Do not pipette reagents by mouth.

4.13. Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.

4.14. Safety Data Sheet for this product is available upon request directly from LTI.

4.15. The Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

5. KIT COMPONENTS

5.1. Contents of the Kit

	Symbol	Description		Qty	Units	Colour code	Stability of opened/diluted components
1	SORB MTP	LH EIA strips, 8x12 wells	polystyrene microwells coated with murine monoclonal to β chain human LH	1	pcs		until exp.date
2	CAL 1–5	Calibrator set, 0.8 ml each, zero calibrator C1 – 2 ml The set contains 5 calibrators: 0; 5; 25; 50; 100 IU/l	human LH diluted in a preselected animal serum preservative – 0,01 % Bronidox L, 0,01 % 2-Methyl-4-isothiazolin-3-one-hydrochloride; also contains red dye	5	pcs	red (C1 – colourless)	2 months
3	CONTROL	Control serum (0.8 ml)	dilution of preselected human serum, with high content of LH with casein solution; preservative – 0,1 % phenol, colourless	1	pcs	colourless	2 months
4	CONJ HRP	Conjugate, 11 ml	aqueous solution of murine monocnoclonal to α chain human LH/FSH/HCG coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0,1 % phenol as preservative and red dye	1	pcs	red	until exp.date
5	SUBS TMB	Substrate solution, 11 ml	ready-to-use single-component tetramethylbenzidine (TMB) solution.	1	pcs	colourless	until exp.date
6	BUF WASH 21X	Washing solution concentrate 21x, 22 ml	aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative	1	pcs	colourless	Concentrate – until exp.date Diluted washing solution – 1 month at +2...+8 °C or 5 days at RT
7	STOP	Stop solution, 11 ml	5,0 % vol/vol solution of sulphuric acid	1	pcs	colourless	until exp.date
8	N003	Plate sealing tape		2	pcs		N/A
9		Instruction LH EIA		1	pcs		N/A
10		QC data sheet LH EIA		1	pcs		N/A

5.2. Equipment and material required but not provided

- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 25–250 µl;
- Dry thermostat for 37 °C ±0.1 °C;
- Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0–3.0

5.3. Storage and stability of the Kit

Store the whole kit at +2...+8 °C upon receipt until the expiration date.

After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

6. SPECIMEN COLLECTION AND STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.

Specimens may be stored for up to 48 hours at +2...+8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/thawing should be avoided.

7. TEST PROCEDURE

7.1. Reagent Preparation

- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18...+25 °C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 21X by 21 dilutions in distilled water.

7.2. Procedural Note:

It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

7.3. Assay flowchart

See the example of calibration graphic in Quality Control data sheet.

7.4. Assay procedure

1	Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators CAL 1–5 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2	If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using (zero calibrator). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.
3	Dispense 100 µl of CONJ HRP into the wells.
4	Pipet 50 µl of calibrators CAL 1–5, control samples CONTROL and unknown samples into the wells. Cover the wells by plate adhesive tape (included into the kit).
5	Incubate 60 minutes at 37 °C .
6	Prepare washing solution by 21x dilution of washing solution concentrate (BUF WASH 21X) by distilled water. Wash the strips 5 times.
7	Dispense 100 µl of SUBS TMB into the wells
8	Incubate 10–20 minutes at +18...+25 °C
9	Dispense 100 µl of STOP into the wells.
10	Measure OD (optical density) at 450 nm.
11	Set photometer blank on first calibrator
12	Apply point-by-point method for data reduction.

8. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

9. CALCULATION OF RESULTS

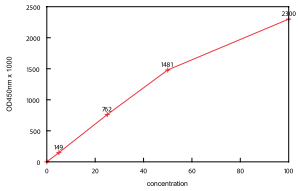
9.1. Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.

9.2. Plot a calibration curve on graph paper: OD versus LH concentration.

9.3. Determine the corresponding concentration of LH in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

9.4. Below is presented a typical example of a standard curve.
Not for calculations!

Calibrators	Value	Absorbance Units (450 nm)
CAL 1	0 IU/l	0.09
CAL 2	5 IU/l	0.24
CAL 3	25 IU/l	0.85
CAL 4	50 IU/l	1.57
CAL 5	100 IU/l	2.39



10. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for LH. Based on data obtained by LTI, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Sex, age	Units, IU/l	
	Lower limit	Upper limit
Children under 11 yrs	1.0	5.0
Males	1.5	9.0
Females		
Menstrual cycle:		
follicular phase	2.0	9.5
luteinic phase	0.5	17
ovulation	10.0	45
post menopausal	5.0	57

11. PERFORMANCE CHARACTERISTIC

11.1. Analytical specificity / Cross reactivity

Analyte	Cross-reactivity, % wt/wt
HCG	<0.1
FSH	<0.1
TSH	<0.1

11.2. Analytical sensitivity

Sensitivity of the assay was assessed as being 0.3 IU/l.

11.3. Linearity

Linearity was checked by assaying dilution series of 5 samples with different LH concentrations. Linearity percentages obtained ranged within 90 to 110 %.

11.4. Recovery

Recovery was estimated by assaying 5 mixed samples with known LH concentrations. The recovery percentages ranged from 90 to 110 %.

12. LITERATURE

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