

DRG® Luteinizing Hormone (LH) ELISA (EIA-1289)

Revised 17 Feb. 2009 (Vers. 6.1)

**1 INTRODUCTION****1.1 Intended Use**

The **DRG LH ELISA Kit** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of the Luteinizing Hormone (LH) in serum.

1.2 Summary and Explanation

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, also called interstitial cell-stimulating hormone (ICSH) in men, is a glycoprotein with a molecular weight of approximately 30.000 daltons (4). It is composed of two non covalently associated dissimilar amino acid chains, alpha and beta (5). 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 (6-8).

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 (9,10). 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 (4). 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 (11).

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 (3,10).

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 (12). Low LH and FSH levels are the result of the negative feedback effects of estradiol and progesterone on the hypothalamic-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 (12).

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 (10,13). A similar loss of regulatory hormones occurs in males when the testes develop abnormally or anorchia exists. High concentrations of LH may also be found in primary testicular failure and Klinefelter syndrome, although LH levels will not necessarily be elevated if the secretion of androgens continues. Increased concentrations of LH are also present during renal failure, cirrhosis, hyperthyroidism, and severe starvation (10,14).

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A lack of secretion by the anterior pituitary may cause lower LH levels. As may be expected, 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 (10).

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.

2 PRINCIPLE OF THE TEST

The DRG LH ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site on a LH molecule. An aliquot of patient sample containing endogenous LH is incubated in the coated well with enzyme conjugate, which is an anti-LH monoclonal antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of LH in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of LH in the patient sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.

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15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H₂SO₄. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

4 REAGENTS

4.1 Reagents provided

1. **Microtiterwells**, 12x8 (break apart) strips, 96 wells
Wells coated with anti-LH antibody (monoclonal)
2. **Standard (Standard 0-5)**, 6 vials (lyophilized), 1 mL
Concentration: 0; 10; 20; 40; 100; 200 mIU/mL.
The standards are calibrated against WHO 2nd International Standard for LH IRP (80/552)
see „Preparation of Reagents“
* contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.
3. **Enzyme Conjugate**, 1 vial, 11 mL, ready to use
Anti-LH antibody conjugated to horseradish peroxidase;
* contains 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.
4. **Substrate Solution**, 1 vial, 14 mL, ready to use
Tetramethylbenzidine (TMB).
5. **Stop Solution**, 1 vial, 14 mL, ready to use
contains 0.5M H₂SO₄
Avoid contact with the stop solution. It may cause skin irritations and burns.

- * BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

Note: Additional *Standard 0* for sample dilution is available on request.

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4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm) (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for six weeks if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Standards

Reconstitute the lyophilized contents of the standard vial with 1 mL Aqua dest.

Note: The reconstituted standards are stable for 2 months at 2 °C to 8 °C. For longer storage freeze at -20 °C.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

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5 SPECIMEN COLLECTION AND PREPARATION

Only serum should be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 48 hours at 2 °C to 8 °C prior to assaying.

Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

- a) dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)
- b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Pipetting of all standards, samples, and controls should be completed within 6 minutes. (Note this especially for manual pipetting.)

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6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **25 µL** of each *Standard, controls* and samples with new disposable tips into appropriate wells.
3. Dispense **100 µL Enzyme Conjugate** into each well.
Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **30 minutes** at room temperature.
5. Briskly shake out the contents of the wells. Rinse the wells **5 times** with aqua dest (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note:

The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

6. Add **100 µL** of *Substrate Solution* to each well.
7. Incubate for **10 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of *Stop Solution* to each well.
9. Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader.
It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 200 mIU/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

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6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 mIU/mL)	0.04
Standard 1 (10 mIU/mL)	0.12
Standard 2 (20 mIU/mL)	0.26
Standard 3 (40 mIU/mL)	0.49
Standard 4 (100 mIU/mL)	1.22
Standard 5 (200 mIU/mL)	1.82

7 EXPECTED VALUES

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

In a study conducted with apparently normal healthy adults, using the DRG LH ELISA the following values are observed:

Population		LH (mIU/mL)
Adult Female	Follicular and luteal phase	≤ 20
	LH Surge	20 – 200
Female, post-menopausal		20 – 100
Males		3 – 12

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

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. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

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In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 1.27 – 200 mIU/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

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

Hormone Tested	Concentration	Produced Color Intensity Equivalent to LH in Serum (mIU/mL)
hCG (WHO 1 st IRP 75/537)	200 mIU/mL	5.2
TSH (WHO 2 nd IRP 80/558)	62 µIU/mL	3.0
FSH (WHO 1 st IRP 68/40)	200 mIU/mL	2.5

NOTE:

Pregnancy results in elevated levels of hCG, the use of the LH enzyme immunoassay test is not recommended during pregnancy or immediately post-partum.

9.3 Sensitivity

The analytical sensitivity of the DRG ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Zero Standard (S0) and was found to be 1.27 mIU/mL.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	1	2	3
Mean (mIU/mL)	2.71	15.72	28.33
SD (mIU/mL)	0.21	0.71	1.29
CV (%)	7.62	4.54	4.57
n =	10	10	10

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9.4.2 Inter Assay

The between assay variability is shown below:

Sample	1	2	3
Mean (mIU/mL)	2.33	15.36	28.96
SD (mIU/mL)	0.26	0.50	1.29
CV (%)	11.02	3.22	4.45
n =	10	10	10

9.5 Recovery

Samples have been spiked by adding LH solutions with known concentrations in a 1:1 ratio.

The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Endogenous LH (mIU/mL)	Added LH (mIU/mL)	Measured Conc. LH (mIU/mL)	Expected * LH (mIU/mL)	Recovery (%)
1	7.7	0	7.7		
		100	98.2	103.9	94.5
		50	55.9	53.9	103.8
		20	25.6	23.9	107.4
		10	12.9	13.9	93.0
2	23.6	0	23.6		
		100	101.7	111.8	90.9
		50	59.9	61.8	96.9
		20	31.0	31.8	97.5
		10	19.8	21.8	90.7
3	52.7	0	52.7		
		100	114.2	126.3	90.4
		50	70.9	76.3	92.8
		20	42.8	46.3	92.2
		10	32.6	36.3	89.7

(* Endogenous LH / 2 + added LH because of a 1:1 dilution of serum with spike material.)

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9.6 Linearity

Sample	Dilution	Measured Conc. (mIU/mL)	Expected Conc. (mIU/mL)	Recovery (%)
1	None	15.6	15.6	
	1:2	7.6	7.8	97.1
	1:4	3.7	3.9	95.7
	1:8	2.0	2.0	101.5
	1:16	0.9	1.0	88.0
2	None	23.6	23.6	
	1:2	11.4	11.8	96.6
	1:4	5.5	5.9	94.0
	1:8	2.6	3.0	89.7
	1:16	1.3	1.5	89.1
3	None	52.7	52.7	
	1:2	25.9	26.3	98.5
	1:4	12.8	13.2	96.9
	1:8	6.8	6.6	103.3
	1:16	3.5	3.3	105.5

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of LH in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 10,000 mIU/mL of LH.

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11 LEGAL ASPECTS

11.1 Reliability 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 national 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. In case of any doubt or concern please contact DRG.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid.

Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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12 REFERENCES

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