Monobind Inc.®
Lake Forest, CA 92630, USA

AccuLite®
CLIA Microwells

Luteinizing Hormone (LH) AccuLite® CLIA Test System

Product Code: 675-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Luteinizing Hormone Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Luteinizing hormone (LH) is a glycoprotein consisting of two subunits with a molecular mass of 30,000 daltons. The α-subunit is similar to other pituitary hormones [follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG)] while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. The α-subunit consists of 89 amino acid residues while the β-subunit contains 129 amino acids. The carbohydrate content is between 15% and 30%.

The clinical usefulness of the measurement of luteinizing hormone (LH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established.[1,2] In addition, the advent of *in vitro* fertilization (IVF) technology to overcome infertility-associated problems has provided the impetus for rapid improvement in LH assay methodology from the technically demanding bioassay[3] to the procedurally simple and rapid immunoenzymometric assays.

In this method, LH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of LH) are added and the reactants mixed. Reaction between the various LH antibodies and native LH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-luteinizing hormone antibody bound conjugate is separated from the unbound enzyme-luteinizing hormone conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable signal to produce light.

The employment of several serum references of known luteinizing hormone levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with luteinizing hormone concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-LH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + Ag_{LH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{LH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{LH} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)} - Ag_{LH} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)} - Ag_{LH} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobolized on well
Immobilized complex = Antibodies-Antigen sandwich bound

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity, determined by reaction with a signal that generates light, in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided

A. LH Calibrators – 1ml/vial - Icons A-F
Six (6) vials of references for LH Antigen at levels of 0(**A**), 5(**B**), 25(**C**), 50(**D**), 100(**E**) and 200(**F**) mIU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IS (80/552).

B. LH Tracer Reagent —13 ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled affinity purified antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Light Reaction Wells – 96 wells – Icon ⇓
One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Signal Reagent A – 7ml/vial - Icon C[A]
One (1) vial containing luminol in buffer. Store at 2-8°C.

F. Signal Reagent B – 7ml/vial - Icon C[B]
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and componenet stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required but Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate luminometer.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirements.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl(of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2 - 30°C.
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****
1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of LH Tracer Reagent solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 45 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
8. Add 0.100 ml (100µl) of working signal reagent to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION
9. Incubate at room temperature in the dark for five (5) min
10. Read the relative light units in each well, for minimum 0.5 – 1.0 seconds, using a microplate luminometer. **The results should be read within thirty (30) minutes of adding the signal solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of hLH in unknown specimens.
1. Record the RLUs (Relative Light Unit) obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLUs for each duplicate serum reference versus the corresponding hLH concentration in mIU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of hLH for an unknown, locate the average RLUs for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (39442) of the unknown intersects the calibration curve at (57.5mIU/ml) LH concentration (See Figure 1).

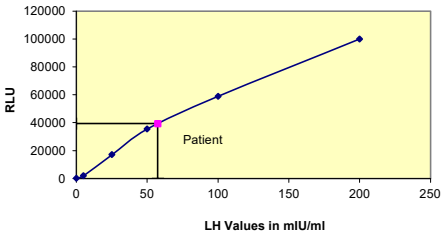
Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (mIU/ml)
Cal A	A1	136	133	0
	B1	129		
Cal B	C1	2011	2035	5
	D1	2060		
Cal C	E1	17289	17072	25
	F1	16855		
Cal D	G1	35680	35228	50
	H1	35376		
Cal E	A2	58911	58986	100
	B2	59062		
Cal F	C2	99818	100000	200
	D2	100182		
Ctrl 1	E2	403	380	**1.0**
	F2	356		
Ctrl 2	G2	9852	9808	**16.2**
	H2	9764		
Patient	A3	38017	39442	**57.5**
	B3	40867		

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. If used, 2 of 3 quality control pools should be within the established range.

12.0 RISK ANALYSIS

The SDS is available at https://www.monobind.com/safety-data-sheets and the Risk Analysis Form may be requested.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
11. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpreataion of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and **should not be the sole basis for therapy**, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. LH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentrations.
8. Luteinizing hormone is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the LH AccuLite® CLIA Test System. The expected values are presented in Table 1.

TABLE I
Expected Values for the LH (in mIU/ml (IRP 68/40))

Women	
Follicular phase	0.5-10.5
Midcycle	18.4-61.2
Luteal phase	0.5-10.5
Postmenopausal	8.2-40.8
Men	
0.7-7.4	

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the LH AccuLite® CLIA Test System were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	0.71	0.06	7.9%
Level 2	20	19.48	0.87	4.5%
Level 3	20	55.30	2.63	4.8%

TABLE 3
Between Assay Precision* (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	0.90	0.09	9.5%
Level 2	20	21.28	2.16	10.1%
Level 3	20	59.53	4.89	8.2%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0mIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. It was determined to be 0.010mIU/ml.

14.3 Accuracy

The LH AccuLite® CLIA Test System was compared with a reference method. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 80. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	13.1	y=0.081+0.975(x)	0.991
Reference	13.8		

Only slight amounts of bias between the LH AccuLite® CLIA Test System and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the LH AccuLite® CLIA Test System to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Luteinizing Hormone needed to produce the same light intensity.

Substance	Cross Reactivity	Concentration
Lutropin (LH)	1.0000	---
β-LH subunit	1.0800	---
Follitropin (FSH)	< 0.0001	1000ng/ml
Chorionic gonadotropin (CG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml

15.0 REFERENCES

1. Kosasa T.S., "Measurement of Human Luteinizing Hormone, *Journal of Reproductive Medicine,* 26, 201-6. (1981)
2. Danzer H., Braunstein G.D., et al., "Maternal Serum Human Chorionic Gonadotropic Concentrations and Fetal Sex Predictions, *Fertility and Sterilit, 34*, 336-40. (1980)
3. Braunstein G.D., et at., "Serum Human Luteinizing Hormone Levels through Normal Pregnancy ", *American Journal of Obstetrics and Gynecology,* 126, 678-81. (1976)
4. Goldstein D.P., and Kosasa T.S., "The Subunit Radioimmunoassay for LH Clinical Application", *Gynecology, 6*, 145-84. (1975)
5. Batzer F., "Hormonal Evaluation of Early Pregnancy", *Fertility and Sterility* 34, 1-12. (1980)
6. Braunstein, G.D., et al., "First-Trimester Luteinizing Hormone Measurements as an Aid to the Diagnosis of Early Pregnancy Disorders", *American Journal of Obstetrics and Gynecology* 131, 25-32. (1978)
7. Lenton E., Neal L. and Sulaiman R., "Plasma Concentrations of Human Gonadotropin from the time of Implantation until the Second Week of Pregnancy *", Fertility and Sterility* 37, 773-78. (1982)

16.0 AVAILABLE CONFIGURATIONS

Available test-system sizes and included materials are shown below. Each "pack size" has a unique item/SKU number, which includes the product code. For the standard pack size of 96-microwells, the product code will have the letter "A" added (e.g., 675-300A) to make a SKU for sale. This test kit is currently available as per below (with contents shown in table):

ITEM #	DESCRIPTION
675-300A	LH AccuLite CLIA Kit - 96 wells
675-300B	LH AccuLite CLIA Kit - 192 wells

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)

Also Available: QSure® Multi-Ligand Control

Revision: 5 **Date: 2025-NOV-14** **DCO: 1747**
MP675.5 **Product Code: 675-300**

For Orders and Inquires, please contact

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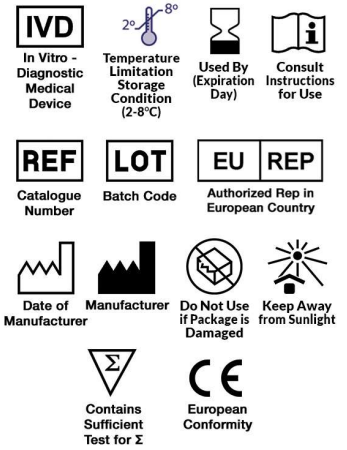
Glossary of Symbols
(ISO 15223)