



## **Luteinizing Hormone (LH) ELISA**

For the quantitative determination of luteinizing hormone (LH) in serum

***Please read carefully due to Critical Changes, e.g., only dilute samples with Calibrator A.***

For “*In Vitro* Diagnostic” use within the United States of America.  
This product is for “Research Use Only” outside of the United States of America.

Catalog Number:	11-LUTHU-E01
Size:	96 wells
Version:	7.0 9/30/2005 - ALPCO 8/17/09

### **ALPCO Diagnostics**

26G Keewaydin Drive • Salem, NH 03079  
Phone: (800) 592-5726 • Fax: (603) 898-6854  
[www.alpco.com](http://www.alpco.com) • Email: [web@alpco.com](mailto:web@alpco.com)

### **INTENDED USE**

For the direct quantitative determination of luteinizing hormone (LH) by enzyme immunoassay in human serum. This kit is for *in vitro* diagnostic use.

### **PRINCIPLE OF THE TEST**

The principle of the following enzyme immunoassay test follows the typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: a monoclonal antibody specific for LH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of LH is conjugated to horse-radish peroxidase (HRP). LH from the sample and standards is allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stop solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of LH in the sample.

A set of standards is used to plot a standard curve from which the amount of LH in patient samples and controls can be directly read.

### **CLINICAL APPLICATIONS**

Human luteinizing hormone (hLH) is a glycoprotein synthesized by the anterior lobe of the pituitary gland. This hormone consists of two subunits:  $\alpha$  and  $\beta$ . The  $\alpha$  subunit of LH is similar to the  $\alpha$  subunit found in both the FSH and TSH glycoprotein hormones (which are also synthesized by the pituitary gland) as well as the  $\alpha$  subunit of hCG (produced by the placenta). However, the  $\beta$  subunits of each of these hormones are unique. Therefore, the specificity of these four hormones is due to the  $\beta$  peptide chains. It is to be noted that the  $\alpha$  chain by itself has no biological activity.

The hypothalamic decapeptide, namely the gonadotropin releasing hormone (GnRH), stimulates the release of LH. Both LH and FSH in men act on the testis, which have two functions: Leydig cells secrete androgens while sperm are formed by the seminiferous tubules. The secretion of testosterone and dihydrotestosterone by the Leydig cells is under the direct control of LH.

### **PROCEDURAL CAUTIONS AND WARNINGS**

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at high and low levels for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human samples.
5. All kit reagents and samples should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and samples.
6. A calibrator curve must be established for every run.
7. The control should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing, as well as improper reagent storage, may be indicated when assay values for the control do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case the substrate solution should not be used.
11. When dispensing the substrate and stop solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

### **LIMITATIONS**

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 sample types of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric, or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit; they may lead to false results.
4. Only calibrator A may be used to dilute serum samples with high LH values. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interference in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.
6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparations of mouse antibody for diagnosis or therapy should be interpreted with caution.

### **SAFETY - CAUTIONS AND WARNINGS POTENTIALLY BIOHAZARDOUS MATERIAL**

Human serum that may be used in the preparation of the standards and control has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However, no test method can offer complete assurance that HIV, HCV, Hepatitis B virus, or any infectious agent are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood sample.

### **CHEMICAL HAZARDS**

Avoid contact with reagents containing TMB, hydrogen peroxide, and sulfuric acid. If contact occurs with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

### **SAMPLE COLLECTION AND STORAGE**

Approximately 0.1 ml of serum is required per duplicate determination. Collect 4-5 ml of blood into an appropriately labeled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human samples potentially biohazardous and take appropriate precautions when handling.

### **SAMPLE PRETREATMENT**

This assay is a direct system; no sample pretreatment is necessary.

### **REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED**

1. Precision pipettes to dispense 25 - 300 µl
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microwell plate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater\* (see assay procedure step 13).

### **REAGENTS PROVIDED**

#### **1. Mouse Anti-hLH Antibody Coated Microwell Plate - Break Apart Wells - Ready To Use.**

Contents: One 96 well (12 x 8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C.

Stability: 12 months or as indicated on label.

#### **2. Mouse Anti-hLH Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.**

Contents: Anti-hLH monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 300 µl/vial

Storage: Refrigerate at 2-8°C.

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:50 in assay buffer before use (e.g., 40 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used, dilute 240 µl of HRP in 12 ml of assay buffer. Discard any remaining volume.

### 3. LH Calibrators - Ready To Use.

Contents: Six vials containing LH in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of LH. Calibrated against World Health Organization (WHO) 2<sup>nd</sup> IS 80/552.

\*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 IU/L	2.0 ml
Calibrator B	1 IU/L	0.5 ml
Calibrator C	4 IU/L	0.5 ml
Calibrator D	10 IU/L	0.5 ml
Calibrator E	40 IU/L	0.5 ml
Calibrator F	100 IU/L	0.5 ml

Storage: Refrigerate at 2-8°C.

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or stored frozen in aliquots. Avoid multiple freezing and thawing cycles.

### 4. Control - Ready To Use.

Contents: One vial containing LH in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of LH. Refer to vial label for expected value and acceptable range.

Volume: 0.5 ml/vial

Storage: Refrigerate at 2-8°C.

Stability: 12 months in unopened vial or as indicated on label. Once opened, the control should be used within 14 days or stored frozen in aliquots. Avoid multiple freezing and thawing cycles.

### 5. Wash Buffer Concentrate - Requires Preparation.

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 ml/bottle

Storage: Refrigerate at 2-8°C.

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

### 6. Assay Buffer - Ready To Use.

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 25 ml/bottle

Storage: Refrigerate at 2-8°C.

Stability: 12 months or as indicated on label.

### 7. TMB Substrate - Ready To Use.

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Volume: 16 ml/bottle

Storage: Refrigerate at 2-8°C.

Stability: 12 months or as indicated on label.

### 8. Stop Solution - Ready To Use.

Contents: One vial containing 1 M sulfuric acid.

Volume: 6 ml/bottle

Storage: Refrigerate at 2-8°C.

Stability: 12 months or as indicated on label.

## ASSAY PROCEDURE

Sample Pretreatment: **None.**

All reagents must reach room temperature before use. Calibrators, controls, and samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the anti-hLH-HRP conjugate and wash buffer.
2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 25  $\mu$ l of each calibrator, control, and sample into the correspondingly labeled wells in duplicate.
4. Pipette 100  $\mu$ l of assay buffer into each well. (It is recommended to use a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
6. Wash the wells 3 times with 300  $\mu$ l of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 100  $\mu$ l of the conjugate working solution into each well. (It is recommended to use a multichannel pipette).
8. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
9. Wash the wells again in the same manner as in step 6.
10. Pipette 100  $\mu$ l of TMB substrate into each well at timed intervals.
11. Incubate on a plate shaker for 15-20 minutes at room temperature (or until calibrator F attains dark blue color for desired OD).
12. Pipette 50  $\mu$ l of stop solution into each well at the same timed intervals as in step 10.
13. Read the plate on a microwell plate reader at 450 nm within 20 minutes after addition of the stop solution.  
\* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower; however, this will not affect the results of the patient/control samples.

## CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the '0' calibrator from the mean absorbance values of the calibrators, control, and serum samples.
4. Draw a calibrator curve on semi-log 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.
5. Read the values of the unknown samples directly off the calibrator curve.

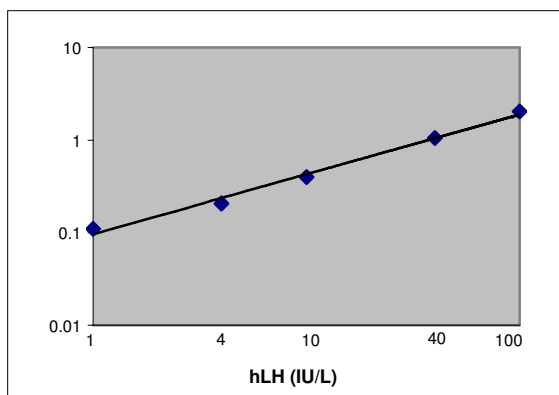
6. If a sample reads more than the value of calibrator F, dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

**TYPICAL TABULATED DATA**

Calibrator	OD 1	OD 2	Mean OD	Value (IU/L)
A	0.080	0.082	0.081	0
B	0.110	0.109	0.110	1
C	0.210	0.203	0.207	4
D	0.388	0.406	0.397	10
E	1.027	1.075	1.051	40
F	2.039	2.049	2.044	100
Unknown	0.527	0.540	0.534	15.6

**TYPICAL CALIBRATOR CURVE**

Sample curve only. **Do not** use to calculate results.



**PERFORMANCE CHARACTERISTICS**

**SENSITIVITY**

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the LH ELISA kit is **0.2 IU/L**.

**SPECIFICITY (CROSS-REACTIVITY)**

The specificity of the assay was determined by measuring the apparent human LH (hLH) value of the following compounds:

Substance	Concentration (IU/L)	Apparent hLH Value (IU/L)
hCG Calibrated against WHO 1 <sup>st</sup> IS 75/537	50,000	55
	25,000	22
	10,000	7.8
	5,000	3.4
	1,000	<1.0
hFSH Calibrated against WHO 1 <sup>st</sup> IS 83/575	1000	13
	500	6.2
	100	1.7
	50	1.5
hTSH Calibrated against WHO 2 <sup>nd</sup> IS 80/558	20	1.2
	500	<1.0
	250	<1.0
	100	<1.0
	50	<1.0
	5	<1.0

**INTRA-ASSAY PRECISION**

Three samples were assayed ten times each on the same calibrator curve. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV%
1	4.84	0.22	4.5
2	16.58	0.44	2.7
3	53.28	1.53	2.9

**INTER-ASSAY PRECISION**

Three samples were assayed ten times over a period of four weeks. The results (in IU/L) are tabulated below:

Sample	Mean	SD	CV%
1	5.15	0.32	5.1
2	17.37	1.40	8.1
3	51.50	4.70	9.2

**RECOVERY**

Spiked samples were prepared by adding defined amounts of human LH to three patient serum samples. The results (in IU/L) are tabulated below:

Sample	Obs. Result	Exp. Result	%Recovery
1 Unspiked	0.00	-	-
+4.9	5.06	4.90	103.3
+48.79	53.79	48.79	110.2
2 Unspiked	2.12	-	-
+3.9	5.76	6.02	95.7
+39.0	40.22	41.12	97.8
3 Unspiked	5.81	-	-
+3.9	9.10	9.71	93.7
+19.5	22.05	25.31	87.1

**LINEARITY**

Two patient serum samples were diluted with calibrator A. The results (in IU/L) are tabulated below:

Sample	Obs. Result	Exp. Result	%Recovery
1	9.28	-	-
1:2	5.02	4.64	108.2
1:4	2.48	2.32	106.9
1:8	1.16	1.16	100.0
2	37.52	-	-
1:2	20.49	18.76	109.2
1:4	10.73	9.38	114.4
1:8	5.44	4.69	116.0
3	42.33	-	-
1:2	20.56	21.17	97.1
1:4	11.20	10.58	105.9
1:8	5.74	5.29	108.5

**HIGH DOSE HOOK EFFECT**

The LH ELISA kit did not experience a high dose hook effect when it was tested up to a human LH concentration of 20,000 IU/L.

## REFERENCE VALUES

As with all clinical assays, each laboratory should collect data and establish its own range of expected normal values.

Group	Range (IU/L)
Males	1.5-9.3
Females	
Follicular Phase	1.9-12.5
Midcycle Peak	8.7-76.3
Luteal Phase	0.5-16.9
Postmenopausal	5.0-52.3

## REFERENCES

1. Hoof, T. D., et al., *J. Clin. Endo. Metab* 57:792, 1983.
2. Ishizuka, B., et al., *J. Clin. Endo. Metab.* 57:111, 1983.
3. Ferrin, M., et al., *Rec. Prag. Horm. Res.* 40:441, 1984.
4. Boyar, R., et al., *N. Engl. J. Med.* 287:287-582, 1972.
5. Cumming, D. D., et al., *J. Clin. Endo. Metab.* 60:810, 1985.
6. Rebar, R., et al., *J. Clin. Invest.* 57:1320, 1976.
7. Cumming, D. C., et al., *Clin. Endocrinol. Suppl.* 177:97, 1973.
8. Simpson E.R., et al., *Ann. Rev. Physiol.* 43:163, 1981.
9. Krieger, D. T., et al., *Biol. Reprod.* 26:55, 1982.
10. Besser, G. M., *J. Endocrinol.* 54:2, 1972.
11. Hunter, W.M., et al., *Alta. Endocrinol. Suppl.* 177:97, 1973.
12. Hosseinian, A.H., et al., *Fert. Steril.* 27:369, 1976.
13. Pavelstein C. J., et al., *An. J. Obstet. Gynoeol.* 130:876, 1978.
14. Kratochwill A., et al., *In Human Ovulation* (Hafexe.S.Ed.) North Holland, Amsterdam, 339, 1979.