

**ELISA kits available from ADI (see details at the web site)**

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)	#0020	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		
#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		
#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta
#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (ft4)
#1650	Human free triiodothyronine (ft3)	#1700	Human T3 (total)
#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnlone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		
#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-0100

## Human Luteinizing Hormone (LH)

**ELISA KIT Cat. No. 0100**

**For Quantitative Determination of LH  
In Human Serum**



*For In Vitro Research Use Only*



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**DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE  
KIT FOR ANY LOT SPECIFIC CHANGES.**

## Human Luteinizing Hormone (LH) ELISA KIT # 0100

For Quantitative Determination of Human LH In Serum

Kit Contents: (reagents for 96 tests)

Components	96 tests
Streptavidin coated strip plate (96 wells), #101	1 Plate
LH Std. A, 0 mIU/mL, 0.5 ml, #102	1 vial
LH Std. B, 5 mIU/mL, 0.5 ml, #103	1 vial
LH Std C, 25 mIU/mL, 0.5 ml, #104	1 vial
LH Std D, 50 mIU/mL 0.5 ml, #105	1 vial
LH Std. E, 100 mIU/mL 0.5 ml, #106	1 vial
LH Std. F, 200 mIU/mL, 0.5 ml, #107	1 vial
Lot specific concn of the standards are given on the vials. Standards are calibrated to WHO 2nd IS 80/552	
Anti-hLH-Enzyme <b>Conjugate</b> , 12 ml, #108	1 bottle
<b>TMB Substrate Solution</b> , 12 ml, # TMB-100	1 bottle
<b>Wash Buffer (20X)</b> ; 25 ml, , #W B - 2 0	1 bottle
<b>Stop</b> solution, 12 ml, # T-10	1 bottle
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### Introduction

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), that is released by the hypothalamus. LH, also called interstitial cell-stimulating hormone (ICSH) in men, is glycoprotein with a molecular weight of approximately 30,000 Dalton. It is composed of two noncovalently 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). LH stimulates ovulation and ovarian steroid production in the female. In the male, LH controls Leydig cell secretion of testosterone. LH is elevated in Luteal phase of menstrual cycle, primary hypogonadism, Gonadotropin-secreting pituitary tumors and menopause. LH is decreased in hypothalamic Gn-RH deficiency, pituitary LH deficiency and ectopic steroid production.

The Analysis of hLH is an important tool in the diagnosis and treatment of infertility in the female. Detection of the hLH surge can aid in predicting the time of ovulation. The onset of the serum hLH surge precedes ovulation by 34 to 35 hours with peak hLH levels occurring several hours later in urine than in serum. The analysis of urinary hLH has been used successfully to time oocyte retrieval for in vitro fertilization and would similarly assist timing of artificial insemination.

### PERFORMANCE CHARACTERISTICS

#### 1. DETECTION LIMIT

Based on sixteen replicates determinations of the zero standard, the minimum concentration of human LH detected using this assay is ~ 0.12 mIU/mL. The detection limit is defined as the value deviating by 2 SD from the zero standard.

#### 2. PRECISION

##### *Intra-assay precision:*

Three serum samples (mean LH concentrations 11.9, 19.5, 46.9 mIU/mL) were run in an assay. The samples showed good intra-assay precision with % CV of 5-620.

##### *Inter-assay precision:*

Three serum samples were run in duplicate in sixteen ten assays. The samples showed good inter-assay precision (4-7% CV). The actual values were: mean 10.30 mIU/mL, SD 1.16 mIU/mL, %CV 7.59; mean 18.40 mIU/mL, SD 1.07 IU/l, %CV 5.84; mean 47.80 mIU/mL, SD 4.16 mIU/mL, %CV 1.9, respectively.

#### 3. LINEARITY

A patient samples (with original LH concentrations 200 mIU/mL) was diluted (1:2, 1:5, and 1:10) with the zero standard and their final LH values determined. The samples showed excellent mean recoveries of about 102% (range 100-105%).

#### 4. HIGH DOSE HOOK EFFECT

LH concentrations of up to 2000 mIU/mL did not show any hook effect.

#### 5. SPECIFICITY

The specificity of LH ELISA kit was determined by measuring interference from high concentrations of hFSH (up to 200 mIU/mL), hTSH (up to 50 uIU/ml), and hCG (25 mIU/mL). These hormones produced color intensity equal to 5, 2.5, and 16 mIU/mL, respectively.

#### 5. INTERFERENCE

The addition of 200 ug/ml each of the following compounds: hemoglobin, atropine, genisic acid, ascorbic acid, acetyl-salicylic acid, in 4 different pool of hLH samples (0, 40, 80, 200

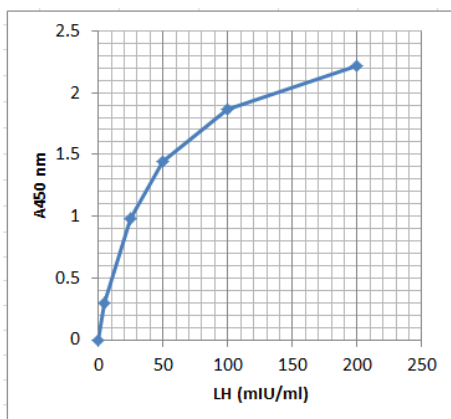
#### 6. SPECIES SPECIFICITY

This kit has not been tested in species other than human. There is substantial sequence conservations of human LH with mouse, rat, monkey LH at the amino acid level. Therefore, antibodies to human LH used in this kit may cross-react with LH from other species but it has not been experimentally verified.

## WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450nm}$
A1, A2	(0 mIU/mL)	0.011
B1, B2	(5 mIU/mL)	0.298
C1, C2	(25 mIU/mL)	0.982
D1, D2	(50 mIU/mL)	1.447
E1, E2	(100 mIU/mL)	1.866
F1, F2	(200 mIU/mL)	2.214
G1, G2	<b>Sample 1</b>	

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

### CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on linear graph paper by plotting net absorbance values of standards against appropriate LH concentrations. Read off the LH concentrations of the control and patient samples.

If ELISA reader software is being used, we recommend 4-parameter or 5-parameter curve.

### PRINCIPLE OF THE TEST

ADI's LH ELISA kit is an adapted solid phase direct sandwich ELISA. The samples, biotin labeled anti-LH and anti-LH-HRP conjugates are added to the wells coated with Streptavidin. The anti-LH Antibodies form a sandwich around LH in the patients serum. Simultaneously, the Biotinylated Anti-LH antibody binds to the Streptavidin coated well. Unbound protein and excess antibody are washed off during a wash step. Upon the addition of the substrate, the intensity of color is proportional to the concentration of LH in the samples. A standard curve is prepared relating color intensity to the concentration of the LH.

### MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100  $\mu$ l) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

### PRECAUTIONS

The Alpha Diagnostic Intl., Inc. LH ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions. All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H<sub>2</sub>SO<sub>4</sub> (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

[http://4adi.com/commerce/info/showpage.jsp?page\\_id=1060&category\\_id=2430&visit=10](http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10)

### SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

### REAGENT PREPARATION:

**Dilute wash buffer (1:20) with distilled water (25 ml stock in 475 ml). Store at 4°C.**

## STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping. Standards are stable for two month at 2-8°C. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

## TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

**Dilute wash buffer (1:20) with distilled water (25 ml stock in 475 ml). Store at 4oC.**

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **25 µl of standards**, control, and serum samples into appropriate wells in *duplicate*.
3. Add **100 µl of enzyme conjugate** into each well. Mix plate by placing on a **plate shaker at 600 RPM** for 30 seconds. Cover the plate and incubate for **60 minutes** at room temperature (18-26 oC)
4. Aspirate and **wash the wells 3 times** with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
5. Dispense **100 ul TMB substrate per well**. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature. Blue color develop in standards and positive wells. **Note:** It is possible to control the color development by increasing or decreasing the incubation time by a 2-5 mins so as to get the maximum A450 of ~2.500. Some ELISA readers may not read above 2.00 then reduce the incubation time.
6. Stop the reaction by adding **50 µl of stop solution** to all wells at the same timed intervals as in step 6. Mix gently. Bluer color turns yellow.
7. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min.

## NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

## DILUTION OF SAMPLES

Serum samples do not usually require dilution. However, if dilution is desired, the zero standard (Sample Diluent) must be used and the results obtained should be multiplied by the appropriate dilution factor.

## EXPECTED VALUES

LH concn of less than or equal to 20 mIU/mL (baseline levels) are normally found during the major portions of follicular and luteal phase of the menstrual cycle. Levels of LH equal to or greater than 40 mIU/mL (40-200) are usually found at the time of hLH surge. Comparison studies between serum and urinary LH concn. throughout the cycle indicate a high degree of correlation between the urinary and serum LH levels starting on day 11 from the initiation of the last menses, which continued through day 16. Peak levels of LH in both the urine and serum were detected on the same day (13-15) following the start of the last menses. The studies indicate that evaluation of urinary LH is just as efficient as serum LH for detecting ovulation in normally cycling females.

## References

Frank JE (1996) J. Pediatr. 128, 548-554; Thakur C (1997) Ind. J. Physiol. Pharmacol. 4, 167-170; Morimoto K (1997) J. Immunol. Methods. 205, 81-90; Maes M (1997) Clin. Endocrinol. 46, 587-598;