9051 Mira Mesa Blvd, #910094
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LH ELISA Kit

Name	LH ELISA Kit
INTENDED USE	The LH ELISA Kit is intended for the quantitative measurement of LH in human serum or plasma.
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), 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 deceased in hypothalamic Gn-RH deficiency, pituitary LH deficiency and ectopic steroid production.
PRINCIPLE OF THE TEST	The LH ELISA kit is a solid phase assay using streptavidin/biotin method. The samples and Anti-LH/Anti-Biotin conjugate are added to the wells coated with Streptavidin. LH in the patient's serum forms a sandwich between specific antibodies labeled with biotin and HRP. Unbound protein and HRP conjugate are washed off by wash buffer. 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 PROVIDED	96 Tests

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Microwells coated with Streptavidin	12x8x1
LH Standard: 6 vials (ready to use)	0.5ml
LH Enzyme Conjugate: 1 bottle (ready to use)	12 ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
Wash concentrate 20X: 1 bottle	25ml

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8 C.

Keep microwells sealed in a dry bag with desiccants.

The reagents are stable until expiration of the kit.

Do not expose test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:

The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.

2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

3. The components in this kit are intended for use as an

	integral unit. The components of different lots should not be mixed. 4. It is recommended that standards, control and serum samples be run in duplicate. 5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
SPECIMEN COLLECTION HANDLING	Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8 C) for 5 days. If storage time exceeds 5 days, store frozen at (-20 C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens.
REAGENTS PREPARATION	Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25C).
ASSAY PROCEDURE	Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use. Place the desired number of coated strips into the holder Pipette 25 l of LH standards, control and patient’s sera. Add 100 l of enzyme conjugate to all wells. Cover the plate and incubate for 60 minutes at room temperature (20-25C). Remove liquid from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbent paper towels. Add 100 l of TMB substrate to all wells. Incubate for 15 minutes at room temperature. Add 50 l of stop solution to all wells. Shake the plate gently to mix the solution. Read absorbance on ELISA Reader at 450 nm within 15

minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

Check LH standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.

To construct the standard curve, plot the absorbance for the LH standards (vertical axis) versus the LH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

EXAMPLE OF STANDARD CURVE

Standard	OD (450nm)
Standard 1 (0 mIU/ml)	0.01
Standard 2 (5 mIU/ml)	0.278
Standard 3 (25 mIU/ml)	0.988
Standard 4 (50 mIU/ml)	1.543
Standard 5 (100 mIU/ml)	2.104
Standard 6 (200 mIU/ml)	2.681

LIMITATIONS OF THE TEST

The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient’s history, physical findings and other diagnostic procedures.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for LH may be used as initial guideline ranges only:

	Male (mIU/ ml)	Female (mIU/ ml)	Female (mIU/ ml)
Adult	1.5-9.3	Follicular phase:	1.9-12.5
Adult	1.5-9.3	Mid-cycle	8.7-76.3
Adult	1.5-9.3	Luteal phase :	0.5-16.9

Adult	1.5-9.3	Post menopausal :	5.0-52.3

PERFORMANCE CHARACTERISTICS

Correlation with a Reference ELISA kit:

A total of 110 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.98	0.9	0.37

2. Precision

Intra-Assay

Serum	No. of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	16	47.2	2.92	6.18
2	16	16.3	1.24	7.60
3	16	1.7	0.18	10.58

Inter-assay

Serum	No. of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	10	46.1	3.75	8.13
2	10	15.6	1.69	10.83
3	10	1.9	0.22	11.57

Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean mIU/ml	Standard Deviation	Mean + 2SD (Sensitivity)
Zero standard	20	0.09	0.015	0.12 mIU/ml

Recovery

Known quantities of LH were added to a serum that contained a low concentration of LH.

Expected Value(mIU/ml)	Recovered (mIU/ml)	Percentage of Recovery
8	8.2	103
16	14.4	90
32	33.5	104

REFERENCES

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