

3. 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	0.008	0.0007	0.0094 mIU/ml

4. 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
1	0.91	103
0.5	0.49	90
0.1	0.09	104

5. Linearity

Two different patient samples were diluted with the "0" calibrator to 1:2, 1:4 and 1:8. LH values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Serum	Original Value (mIU/ml)	Percentage of Recovery		
		1:2	1:4	1:8
1	1	96	98	89
2	8	109	93	91

6. Cross Reactivity

The LH lumELISA cross-reactivity was evaluated by adding the interfering substances to a serum matrix at various concentrations. The cross reactivity was calculated by deriving a ratio between dose response of the interfering substance to dose of LH needed to produce the same light intensity.

Substance	Cross Reactivity	Concentration (ng/ml)
LH	1	
hFSH	<0.0001	500
hCG	<0.00005	500
TSH	<0.0001	500

REFERENCES:





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For Research Use Only. Not for use in Diagnostic Procedures.

Cat#: LH550F, (96 Tests)
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Ultra Sensitive Luteinizing Hormone (LH) lumELISA

Catalog No. LH550F, (96 Tests)

INTENDED USE

The Calbiotech, Inc. (CBI) Ultra Sensitive LH lumELISA (Chemiluminescence Enzyme Linked Immunosorbent Assay) is used for the ultra sensitive 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 decreased in hypothalamic Gn-RH deficiency, pituitary LH deficiency and ectopic steroid production.

In children, abnormalities in concentration of LH can be aid in the diagnosis of pituitary disorders, and may be indicative of problems in the reproductive system of both genders, infertility problems, early and delay puberty.

PRINCIPLE OF THE TEST

The Ultra Sensitive LH lumELISA kit is based on the streptavidin and biotin principle. 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 the 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. Simultaneously, the complex is deposited to the well through the high affinity reaction streptavidin and biotinylated antibody. After one hour incubation, unbound protein and conjugates are washed off by wash buffer. Upon the addition of the substrate, the enzyme activity in the enzyme-bound fraction is directly proportional to the concentration of LH in the samples. A standard curve is prepared relating light units to the concentration of the LH.

MATERIALS PROVIDED	96 Tests
Microwells coated with Streptavidin	6x8x2 wells
LH Standard: 7 vials (ready to use)	0.4 ml
Biotin Conjugate , 20X: 1 Bottle	0.85 ml
Enzyme Conjugate, 20X: 1 Bottle	0.85ml
Assay Diluent, 1 bottle (ready to use)	12 ml
Luminol Substrate, 3X: 1 Bottle	5 ml
Luminol Buffer: 1 Bottle	10 ml
Wash Concentrate, 20X: 1 Bottle	25 ml

MATERIALS NOT PROVIDED

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- Microplate Luminometer
- Absorbance paper or paper towel
- Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. 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. This test kit is USA FDA exempt product. For In Vitro Diagnostic Use.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. 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

1. Collect blood specimens and separate the serum immediately.
2. 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.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

1. 20X Biotin Conjugate and 20X Enzyme Conjugates: Prepare 1X working dilution at 1:20 with assay diluent as needed, e.g. 0.1 ml from each stock conjugate in 1.8 ml of assay diluent is sufficient for 20 wells. The diluted conjugate has to be used the same day.
2. 20X Wash Buffer Concentrate: Prepare 1X wash buffer by adding the contents of the bottle to 475 ml of distilled water. Store 1X wash buffer at room temperature.
3. 3X Luminol Substrate: Prepare 1X Substrate solution by adding 1 part of Luminol to 2 parts Luminol buffer as needed.

ASSAY PROCEDURE

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Add 25 µl of LH standards, control and patient's sera into selected wells.
3. Add 100 µl of 1X Biotin/Enzyme Conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18-26° C) with shaking.
5. Remove liquid from all wells. Wash wells five times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of 1X Luminol substrate to all wells.
Read the relative light units in each well using Luminometer (0.2-1 second integration time) within 5 min of substrate addition.

Note: Loss of sensitivity in low range standards may be observed if the wait time is more than 10 minutes after adding the substrate.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. 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.
2. To construct the standard curve, plot the RLU (Relative Light Units) for each LH standard point (vertical axis) versus the LH standard concentrations (horizontal axis) on a log graph paper. Draw the best curve through the points.

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

Example of a Standard Curve

Standard	RLU
Standard 1 (0 mIU/ml)	3794
Standard 2 (0.05 mIU/ml)	10953
Standard 3 (0.2 mIU/ml)	32556
Standard 4 (1 mIU/ml)	154771
Standard 5 (2 mIU/ml)	300064
Standard 6 (10 mIU/ml)	1882709
Standard 7 (50 mIU/ml)	9467393

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)
Adult	1.5-9.3	Follicular phase: 1.9-12.5
		Mid-cycle : 8.7-76.3
		Luteal phase : 0.5-16.9
		Post menopausal : 5.0-52.3
Children		
Cord Blood	0.04-2.60	0.04-2.60
2 weeks	4.85-10.02	0.29-7.91
1-18 months	0.04-3.01	0.02-1.77
19 months – 7 y	0.02-1.03	0.03-0.55
8-9 y	0.01-0.78	0.02-0.24
10-11 y	0.03-4.44	0.02-4.12
12-14 y	0.25-4.84	0.28-29.38
15-18 y	0.69-7.15	0.11-29.38

LIMITATIONS OF THE TEST

1. 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.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS

1. **Correlation with a Reference lumELISA kit:**

A total of 74 sera were tested by this lumELISA and a reference LIA kit. Results were as follows:

Correlation	Slope	Intercept
0.97	0.9	0.34

2. **Precision**

Intra-Assay

Serum	No. of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	16	24.0	1.36	5.67
2	16	9.70	0.83	8.56
3	16	0.33	0.03	8.98

Inter-Assay

Serum	No. of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	10	25.9	1.76	6.80
2	10	10.2	0.83	8.14
3	10	0.47	0.04	8.51