OriGene Technologies, Inc.
9620 Medical Center Dr., Suite 200, Rockville, MD 20850
Phone: 1.888.267.4436 Fax: 301-340-9254
Email: techsupport@origene.com Web: www.origene.com

Product Information

Ultra Sensitive Luteinizing Hormone (LH) lumELISA kit

Catalog Number: EA100845
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Ultra Sensitive LH lumELISA™ is used for the ultra-sensitive quantitative measurement of LH in human serum or plasma.

Background

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. 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.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	6x8x2 wells
2. LH Standard: 7 vials (ready to use)	0.2 ml
3. Biotin Conjugate , 20X: 1 Bottle	0.7 ml
4. Enzyme Conjugate, 20X: 1 Bottle	0.7 ml
5. Assay Diluent, 1 bottle (ready to use)	12 ml
6. Luminol Substrate, 3X: 1 Bottle	4 ml
7. Luminol Buffer: 1 Bottle	8 ml
8. Wash Concentrate, 20X: 1 Bottle	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

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.

Reagent 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

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.

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

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.
7. Read the relative light units in each well using Luminometer (0.2-1 second integration time) within 5 min of substrate addition.

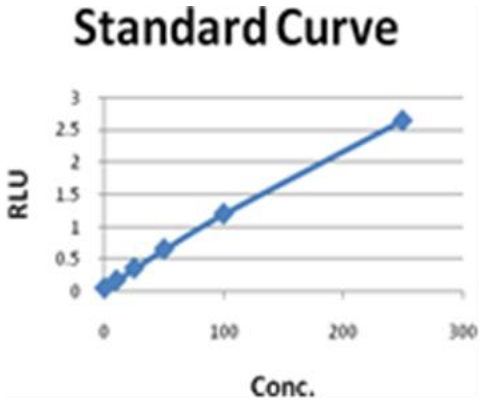
Calculation of Results

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	Male (mIU/ ml)	Female (mIU/ ml)
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

References

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2. Frank JE; Faix JE; Hermos RJ; Mullaney DM; Rojan DA; Mitchell ML; Klein RZ Thyroid function in very low birth weight infants: effects on neonatal hypothyroidism screening. J Pediatr 1996;128(4):548-54.
3. Thakur C; Saikia TC; Yadav RN. Total serum levels of triiodothyronine (T3) thyroxine (T4) and thyrotropine (LH) in school going children of Dibrugarh district: an endemic goitre region of Assam. Indian J Physiol Pharmacol 1997;41(2):167-70.
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