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Product Information

Luteinizing Hormone (LH) ELISA kit

Catalog Number: EA100844
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use
The LH ELISA Kit is intended for the quantitative measurement of LH in human serum.

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.

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.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. LH Standard: 6 vials (ready to use)	0.5ml
3. LH Enzyme Conjugate: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12ml
5. Stop Solution: 1 bottle (ready to use)	12ml
6. Wash concentrate 20X: 1 bottle	25ml

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

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 (18-26°C).

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. Pipette 25 µl of LH standards, control and patient's sera.
3. Add 100 µl of enzyme conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18-26°C).
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

Calculation of Results

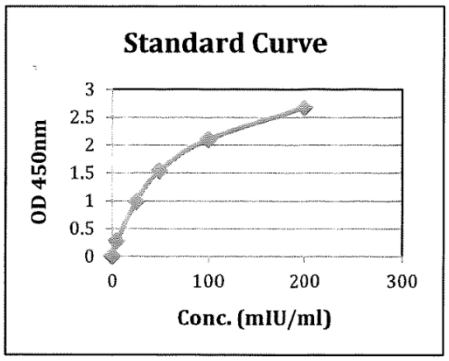
The standard curve is constructed as follows:

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

Example of a Standard Curve

Standard	Conc (mIU/ml)	OD450 nm
Std 1	0	0.01
Std 2	10	0.278
Std 3	25	0.988
Std 4	50	1.543
Std 5	100	2.104
Std 6	250	2.681



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

References

1. 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.
2. 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.
3. Morimoto K; Inouye K.A sensitive enzyme immunoassay of human thyroid-stimulating hormone (LH) using bispecific F(ab')2 fragments recognizing polymerized alkaline phosphatase and LH. J Immunol Methods 1997;205(1):81-90.
4. Maes M; Mommen K; Hendrickx D; Peeters D; D'Hondt P; Ranjan R; De Meyer F; Scharp´e S. Components of biological variation, including seasonality, in blood concentrations of LH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clin Endocrinol (Oxf) 1997;46(5):587-98.

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