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Luteinizing Hormone (LH) ELISA

Catalog No.: IB19104 (96 Tests)

INTENDED USE

The LH ELISA Kit is intended for the measurement of LH in human serum or plasma. For research use only – Not for use in diagnostic procedures.

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

MATERIALS 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

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 kit is designed for research use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which samples or kit reagents are handled.
4. Components in this kit are intended for use as an integral unit. 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.

SAMPLE COLLECTION HANDLING

1. Collect blood samples and separate the serum immediately.
2. Samples 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 samples.

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 (18-26° C).

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. Pipette 25 µl of LH standards, control and sample.
3. Add 100 µl of Conjugate Reagent to all wells. Mix plate by placing on a plate shaker at 600rpm for 30 seconds.
4. 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.

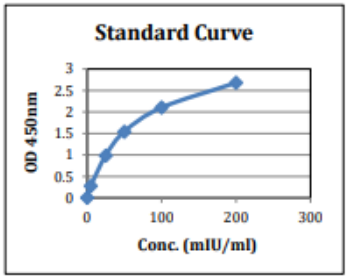
CALCULATION OF RESULTS

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 Standard Curve

Standard	OD (450 nm)
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

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