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Calbiotech, Inc.
1935 Cordell Ct., El Cajon, CA 92020 USA
Tel (619) 660-6162 | Fax (619) 660-6970 | Web www.calbiotech.com

CALBIOTECH

Luteinizing Hormone (LH) ELISA

Catalog No.: LH231F (96 Tests)

INTENDED USE

The Calbiotech LH ELISA Kit is intended for the quantitative measurement of LH in human serum or plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Luteinizing hormone is a gonadotropin that triggers ovulation and stimulates testosterone production. This assay quantifies LH for research on reproductive endocrinology, gonadotropin-releasing hormone pathways, and hormonal cycle regulation.

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

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

Potential biohazardous materials:

1. The standards 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, as 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

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

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 (20-25° 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 patient's sera.
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 (20-25°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 (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

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