Human Luteinizing Hormone （LH） ELISA Test Kit

NAME AND INTENDED USE

Detection Kit for Human Luteinizing Hormone（LH）(Enzyme-Linked ImmunoSorbent Assay , ELISA).It is used in quantitative tests for luteinizing hormone in human serum.

SUMMARY AND CLINICAL SIGNIFICANCE

Human Luteinizing Hormone (LH) is secreted by the □-cells of the anterior pituitary under the control of hypothalamic gonadotropin releasing hormone (GnRH). LH in the female causes ovulation and steroid (estrogen and progesterone) production by the corpus luteum. In the male it stimulates interstitial cells (Leydig cells) to produce androgens and estrogens. Circulating levels of LH are controlled by a negative feedback effect on the hypothalamus by the steroid hormones. In sexually mature adults, FSH and LH are not secreted in constant amounts; rather, secretion occurs in pulses which result in rapid fluctuations over the entire reference range (up or down by 50 to 100%). Because of this pulsatile secretion, samples obtained in a single day from the same patient may fluctuate widely within the reference range, reflecting physiological variation rather than errors in technique or methodology.The primary clinical use of LH measurement is in clearly defining the hypothalamic-pituitary-gonadal axis. Measurement of serum gonadotropin levels will allow for distinguishing between primary gonadal failure and deficient gonadal stimulation. If LH and FSH levels are elevated (hypergonadotropic hypogonadism), primary gonadal failure is present. If, on the other hand, gonadotropin levels are low (hypogonadotropic hypogonadism), deficient gonadal stimulation has resulted in the hypogonadal state.LH measurement is also of clinical importance because growth hormone and LH are frequently the first hormones to be affected by pituitary disease.Serum determinations have been very useful in the diagnosis and treatment of infertility in women. A midcycle rise in the LH level is a good indication that ovulation will occur approximately 24 hours later. The reproductive phase in females is terminated by menopause, due to low levels of circulating estradiol and progesterone, there is a loss of negative feedback to the hypothalamus; as a result, circulating levels of LH are greatly increased. Similarly, LH levels are increased in younger women of premenopausal age who suffer ovarian failure or whose ovaries have failed to develop during puberty. It is important to note that the midcycle peak is completely obliterated in healthy women using oral contraceptives. Testosterone and estrogen administration depress LH levels in the post-menopausal state.

PRINCIPLE

LH uses a “sandwich principle”, Enzyme-linked immunological sorbent assay. To measure LH levels in serum, plastic wells coated with a monoclonal antibody of LH are supplied in the kit. After the patient’s specimen and another mono-antibody labelled with HRP are added, LH, if present, is fixed to the solid phase antibody and creating a HRP-antibody—LH—antibody “sandwich”. After TMB substrate added, the result is obtained by EIA plate reader.

PRECAUTION FOR USERS

1. Handling should preclude any pipetting by mouth.
2. Use only pipettes with disposable tips for each specimen.
3. Do not mix materials from different master lots. Do not use kit components beyond the expiration date. All materials should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

Serum specimens can be tested by the LH procedure. Remove serum from the clot as soon as possible to avoid hemolysis. Covered specimens can be stored up to 48 hours at 2~8℃. Specimens held for a longer time can be frozen at –20°C and avoid repeated freeze melting. Serum samples with concentrations expected to be greater than 75IU/L should be diluted with normal saline.

NOTE: If needed, remove by centrifugation the suspended fibrin particles or aggregates which are liable to produce falsely positive results.

REAGENTS SUPPLIED

1. Coated Microplate: 1 plate (8×12 wells), Ready to use. Coated with anti-LH antibody and sealed in an aluminum bag. Remove the strips in the resealable bag with a desiccant to protect from moisture after opened. Store at 2~8℃ until expiration date.
2. HRP Conjugate: 1 vial of 11ml, Ready to use. Store at 2~8℃ until expiration date.
3. Antibody: 1 vial of 6ml, Ready to use. Store at 2~8℃ until expiration date.
4. Calibrator: 6 vials of 0.5ml, Ready to use. Labeled with S0 to S5 and the concentration of LH is 0, 1, 3, 9, 25, 50 IU/L. Store at 2~8℃ until expiration date.
5. Control: 2 vials of 0.5ml, Ready to use. The concentration of low value is 2.4~4.4IU/L. The concentration of high value is 6.9~12.8IU/L. Store at 2~8℃ until expiration date.
6. Chromogen A: 1 vial of 7ml, Ready to use. Store at 2~8℃ until expiration date.
7. Chromogen B: 1 vial of 7ml, Ready to use. Store at 2~8℃ until expiration date.
8. Stop Solution: 1 vial of 7ml, Ready to use. Store at 2~8℃ until expiration date.
9. Wash buffer: 1 vial of 15ml, Concentrate 20-fold, diluting with deionized water before the assay. Store at 2~8℃ until expiration date.
10. Plate sealer: 2 pieces.
11. Plastic resealable bag: 1 set.
12. Instruction manual: 1 copy.

RELATED TIPS

1. This kit is designated for In-Vitro Diagnostic Use Only.
2. Wash procedure. Incomplete washing will adversely affect the test results. Wash each well 3 times with about 0.3ml diluted washing solution. If no automatic washer is available, washing can be performed manually as follows: Invert the plate vigorously to get all water out and block the rim of well on absorbent paper for a few seconds. Filling each well with diluted washing solution and remain 10 seconds. Repeat these steps 3 times. Blot dry the plate by inverting the plate onto absorbent tissue, and striking a hard surface several times.
3. Drip procedure. Mix the bottle gently before use. Violent surge may cause too much foam. Invert the bottle and squeeze one or two drop on absorbent tissue to make sure there is no foam. Take the bottle upright the well and make sure the drop does not touch the rim of wells.
4. Storage. The whole kit should be stored at 2~8℃ for one year. Microplate should be taken to room temperature before opened. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. After removing the required number of strips, the plate should be put in the plastic resealable bag with desiccants to minimize exposure to damp air.
5. Read Procedure. Determine the absorbance (OD) of each well at 450nm with a microtiter plate reader. Using the blank well to correct the zero point of reader if single wavelength reader is used. If double wavelength readers with 450nm and 630nm are used, there is no need to correct the zero point.
6. Control serum is prepared with human serum, which is tested negative of HBV, HCV and HIV. But it should still be considered as capable of transmitting viral diseases.

PREPARATIONS

1. Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use.
2. Prepare Wash buffer by diluting Wash Concentrate 20-fold with deionized water. The diluted wash solution is stable in room temperature for at least one week.

ASSAY PROCEDURE:

1. Mark the microtitration strips to be used. All the Calibrators and controls should set duplicate.
2. Dispense 50 ul of calibrators / controls / samples into respective wells.
3. Dispense 50 µl of Antibody to each well
4. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 1 hour.
5. Wash each well for 3 times, 10 seconds each time. (See wash procedure).
6. Dispense 100 ul of HRP Conjugate to each well
7. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 30 minutes.
8. Wash each well for 3 times, 10 seconds each time. (See wash procedure).
9. Dispense 50 ul of chromogen A to each well.
10. Dispense 50 ul of chromogen B to each well.
11. Covered the strips with a fresh plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 15 minutes.
12. Dispense 50 ul of stop solution to each well and mix completely.
13. Read the absorbance of the plate within 10 minutes. (See read procedure)

CALCULATION OF RESULTS

Computer: Use the linear fitting function, the logarithm of each calibrator concentration (Log), as X, take the logarithm of the corresponding absorbance value (Log(OD)) as Y, choose double logarithm (or full Logarithmic) Log-Log fitting the concentration of the serum to be tested is calculated from the fitted line.

Equation: log OD=B*log [concentration]+A

PERFORMANCE CHARACTERISTICS

1. Expected value

The cut-off value of LH concentration for healthy subjects is:

Male: 1.08~8.34 IU/L;

Famale: follicular phase 1.0~18.54 IU/L;

corpus luteum phase 1.0~21.55 IU/L;

ovulation phase 4.8~72.2 IU/L;

menopause 9.14~58.32 IU/L l.

Sensitivity

The detection limit of the assay is approximately 0.5 IU/L.

2. Precision

Interassay≤15%

Intraassay≤15%

3. Specificity: No cross reaction with TSH, FSH, HCG.

CODIGO: RSET120-3