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User's Manual

LH (Urine) ELISA Kit

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Leutenizing Horome (LH) ELISA is an enzyme immunoassay for the quantitative measurement of LH in urine. This test is used to detect the midcycle LH surge in urine, which is an aid in predicting the time of ovulation.

General Description

Luteinizing Hormone (LH) is a crucial heterodimeric glycoprotein hormone produced and secreted by the gonadotropic cells of the anterior pituitary gland, under the regulation of Gonadotropin-Releasing Hormone (Gn-RH) from the hypothalamus. In men (where it is also called ICSH), LH primarily stimulates the Leydig cells in the testes to produce testosterone. In women, LH plays a central role in the menstrual cycle, where a dramatic rise known as the "LH surge" triggers ovulation and initiates the formation of the corpus luteum, which subsequently secretes progesterone and estrogen. LH assays are routinely used in clinical settings, often alongside FSH, to evaluate pituitary and gonadal function, pinpoint ovulation, and diagnose conditions like menopause.

Principles of Testing

The Luteinizing Hormone (LH) ELISA Kit is a quantitative, solid-phase immunoassay based on the sandwich principle. The assay utilizes microtiter wells coated with a specific monoclonal antibody directed against the β-subunit of the LH molecule, which captures the analyte from the patient sample during the initial incubation. Following this capture, an enzyme conjugate—an anti-LH antibody linked to Horseradish Peroxidase (HRP)—is added to form an antibody-LH-conjugate "sandwich" complex. After a stringent wash to remove unbound material, a substrate solution is introduced. The HRP catalyzes a colorimetric reaction which is then stopped, producing a final yellow product. The intensity of this color, measured spectrophotometrically as Optical Density (OD), is directly proportional to the LH concentration in the sample, allowing the final quantitative determination through interpolation on a reference standard curve.

Reagents And Materials Provided

1. Pre-coated 96-Well Plate: 12×8 (break apart) strips
2. Standard: 1 mL × 6 (0, 10, 20, 40, 100, 200 mIU/mL)
3. Enzyme Conjugate: 11 mL × 1
4. Substrate Solution: 14 mL × 1
5. Stop Solution: 14 mL × 1
6. Wash Solution (40×): 30 mL × 1

Materials Required But Not Supplied

1. A calibrated microtiter plate reader (450, with reference wavelength at 620 nm to 630 nm))

2. Calibrated variable precision micropipettes
3. Absorbent paper
4. Distilled water
5. Timer
6. Semi-logarithmic graph paper or software for data reduction

Storage

When stored at 2 °C to 8 °C, unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for four weeks if stored as described above.

Specimen Collection And Preparation

This kit is strictly for use with urine samples only that contain no additives or preservatives.

1. Sample Collection

It is recommended to collect first morning urine (after fasting). First clean genital area with mild disinfectant to prevent contamination. Then collect clean-catch midstream urine in an appropriate sterile container without preservatives.

Directly after collection, the urine should be centrifuged for 5 to 10 minutes (e.g., at 2000 xg) to remove cellular debris. Use supernatant for analyte quantification.

Note: The variation in LH concentrations in women is subject to the complex ovulatory cycle of healthy menstruating women.

2. Sample Storage and Preparation

The urine supernatant should be capped and may be stored for up to 7 days at 2 °C to 8 °C prior to assaying. The supernatants cannot be stored frozen.

Reagent Preparation

Bring all reagents and required number of strips to room temperature (20°C to 26°C) prior to use.

Standards

Reconstitute the lyophilized contents of each vial with 1mL distilled water and let stand for at least 10 minutes at room temperature. Mix several times before use.

The reconsitituted standards are stable for 4 weeks at 2 °C to 8 °C. For longer storage (up to 18 months), aliquot and freeze reconstituted standards at -20°C.

Wash Solution

Add distilled water to the 40× concentrated Wash Solution. Dilute 30 mL of concentrated Wash Solution with 1170 mL distilled water to a final volume of 1200 mL. The 1× working Wash Solution is stable for 1 week at room temperature.

Assay Procedure

General Remarks

1. All reagents and samples must be allowed to reach room temperature and should be mixed gently, avoiding foam formation, before use.
2. Once the test procedure has commenced, all steps must be carried out without interruption to ensure consistency.
3. Utilize a new disposable plastic pipette tip for each standard, control, or sample to prevent cross-contamination.
4. Since the final absorbance is dependent on incubation time and temperature, it is highly recommended to prepare all materials (reagents, removed caps, wells secured) before starting to ensure equal elapsed time for every pipetting step.
5. In general, the enzymatic reaction rate demonstrates a linear relationship with both time and temperature.

Assay Procedure

Note: A standard curve must be included in each run for quantification.

1. Secure the required number of microtiter wells within the frame holder.
2. Using new tips, dispense 50 µL of each Standard, control, and sample into the appropriate wells.
3. Incubate the plate for 30 minutes at room temperature.
4. Dispense 100 µL of Enzyme Conjugate into every well. It is essential to mix thoroughly for 10 seconds at this stage.
5. Incubate for an additional 30 minutes at room temperature.
6. Wash Step: Vigorously shake out the well contents. Rinse the wells three times with 300 µL of 1× Working Wash Solution per well. Blot the plate sharply on absorbent paper to remove all residual droplets. (Important: Accurate washing is crucial for the assay's sensitivity and precision.)
7. Add 100 µL of Substrate Solution to each well.
8. Incubate for 10 minutes at room temperature (color development).
9. Stop the enzymatic reaction by dispensing 50 µL of Stop Solution into every well.
10. Reading: Determine the absorbance (OD) of the wells using a plate reader at 45 nm (primary reading) and, if recommended, at 620 nm to 630 nm (for background subtraction). It is advisable to read the plate within 10 minutes of adding the Stop Solution.