EZElisa™ Human Luteinizing Hormone (LH) ELISA Kit

Cat #: D-AEK6902

Size: 48T / 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Detection range: 2 IU/L-75 IU/L

Sensitivity: 0.5 IU/L

Specificity: EZElisa™ Human LH ELISA Kit has high sensitivity and excellent specificity for detection of Human LH. No significant cross-reactivity or interference between Human LH and analogues was observed

Applicable samples: Serum, Plasma

Assay Principle

Luteinizing hormone (LH) is produced in both men and women from the anterior pituitary gland in response to luteinizing hormonereleasing 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. 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. EZElisa™ Human LH ELISA Kit employs a two-site sandwich ELISA to quantitate Human LH in samples. An antibody specific for Human LH has been pre-coated onto a microplate. Standards and samples are added to the appropriate microtiter plate wells with HRP conjugated antibody specific for Human LH and any Human LH present is bound by the immobilized antibody. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Human LH in the sample. The color development is stopped and the intensity of the color is measured.

Materials Supplied and Storage Conditions

Kit components	Size（48T）	Size（96T）	Storage conditions
Human LH Standard (lyophilized)	1×6	1×6	4°C
HRP conjugated Human LH Detect Antibody	3 mL	6 mL	4°C
HRP Substrate A	3.5 mL	7 mL	4°C,protected from light
HRP Substrate B	3.5 mL	7 mL	4°C,protected from light
Stop Solution	3.5 mL	7 mL	4°C
Wash Buffer (20×)	7.5 mL	15 mL	4°C
Human LH Microplate	48 wells	96 wells	4°C
Plate Covers	1	2	RT

Note: Std1: 0 IU/L; Std2: 2 IU/L; Std3: 5 IU/L; Std4: 20 IU/L; Std5: 40 IU/L; Std6: 75 IU/L.

Materials Required but Not Supplied

·Microplate reader capable of measuring absorbance at 450 nm

·Multi channel pipette or automated microplate washer

·Incubator, refrigerated centrifuge

·Precision pipettes, disposable pipette tips

·Deionized water

Reagent Preparation

Note: Bring all reagents equilibrate to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

1×Wash Buffer: Wash buffer(20×) dilute with deionized water 1:20 to obtain the 1×Wash Buffer. Store at 4°C.

Human LH standard: Add 700 µL of Deionized water into the lyophilized standard vial. Dissolve the powder thoroughly by gentle shake.

Sample Preparation

1.Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

2.Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Note: Do not use grossly hemolyzed or lipemic specimens Samples should be aliquoted and must be stored at -20°C to avoid loss of bioactive Human LH. If samples are to be used within 24 hours, they may be stored at 2 to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

2. Add 50 µL of Human LH Standard or Sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Set a Blank well without any solution.

3. Add 50 µL of HRP conjugated Human LH detect antibody to each well (not to Blank well). Mix well, cover with the plate cover provided and then incubate for 1 h at 37°C.

4. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 µL) using a Multi channel pipette or automated microplate washer, and let it stand for 10 s, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.

5. Add 50 µL of Substrate A and 50 µL of Substrate B to each well, mix well and cover with the plate cover provided. Incubate for 15 min at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

6. Add 50 µL of Stop solution to each well. Stop Solution should be added to the plate in the same order as HRP substrate. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1.Average the duplicate readings for each standard and sample and subtract the average zero standard (Std1) optical density (O.D.).

2.Drawing of standard curve: With the standard solution concentration as the x-axis and the mean optical density (O.D.) for each standard as the y-axis, draw the standard curve. A computer software can be used to create a standard curve.

Typical Data

Typical standard curve （R^2≥0.99）

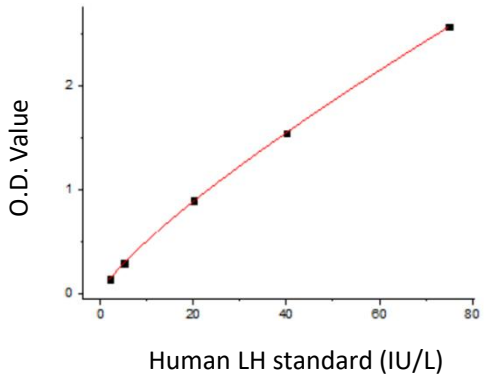

Fig.1. Standard Curve of Human LH in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay

Precautions

1.Do not mix or substitute reagents with those from other lots or sources.

2.To avoid cross-contamination, change pipette tips between additions of each standard level, between sample

additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.

4. Stop Solution has certain Corrosive. Please take protective measures when operating.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.