EZElisa™ Human Follicle Stimulating Hormone (FSH) ELISA Kit

Cat #: D-AEK6903

Size: 48T / 96T

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

Product Information

Detection range: 2 IU/L-70 IU/L

Sensitivity: 1.0 IU/L

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

Applicable samples: Serum, Plasma

Assay Principle

Follicle Stimulating Hormone (FSH) is a glygoprotein produced by the anterior pituitary gland. In the female, FSH stimulates follicular growth, prepares ovarian follicles for action by LH and enhances the LH induced release of estrogen. FSH levels are elevated after menopause, castration and in premature ovarian failure. Although there are significant exceptions ovarian failure is indicated when random FSH concentrations exceed 40 mIU/ml. In the male, FSH stimulates seminiferous tubule and testicular growth and is involved in the early stages of spermatogenesis. Oligospermic males usually have elevated FSH levels. Tumors of the testes generally depress serum FSH concentrations, but levels of LH are elevated. High levels of FSH in men may be found in primary testicular failure and Klinefelter syndrome. Elevated concentrations are also present in cases of starvation, renal failure, hyperthyroidism, and cirrhosis. EZElisa™ Human FSH ELISA Kit employs a two-site sandwich ELISA to quantitate Human FSH in samples. An antibody specific for Human FSH 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 FSH and any Human FSH 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 FSH 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 FSH Standard	0.25 mL×6	0.5 mL×6	4°C
HRP Conjugated Human FSH 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 FSH 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: 10 IU/L; Std5: 25 IU/L; Std6: 70 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.

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 FSH 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 FSH 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 mean optical density (O.D.) for each standard as the x-axis and the standard solution concentration 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）

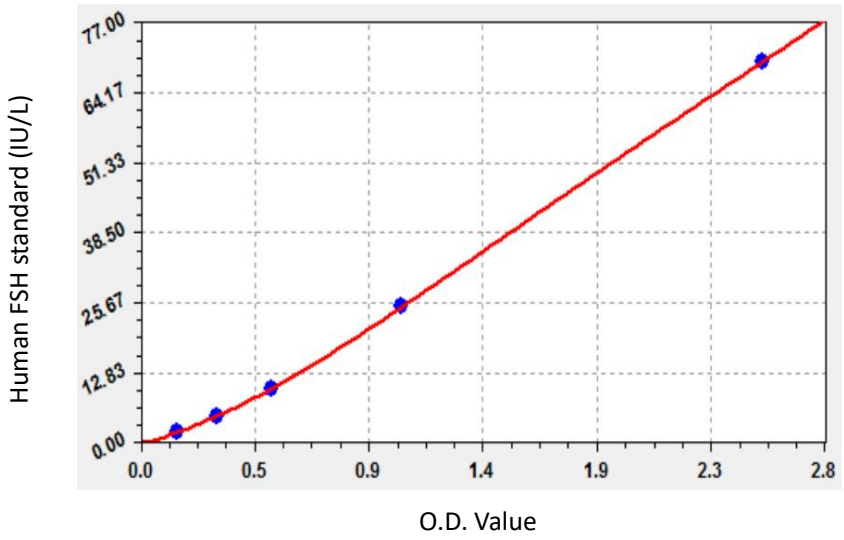

Fig.1. Standard Curve of Human FSH in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay

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.