Human FSH ELISA Kit

to quantitatively determine Human FSH in Human Blood Serum or Plasma

INSTRUCTION MANUAL

FOR ELISA KIT No: LTHS302K

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INTENDED USE

This kit is used to quantitatively determine the Human FSH in the sample of Human Blood Serum or Plasma. For *in vitro* use only.

MANUAL VERSION 1.02X2

BACKGROUND

Follicle stimulating hormone (FSH) is a glycoprotein with molecular weight 28 kDa secreted by basophil cells in hypophysis. Gonadotropin releasing hormone (GnRH) produced by the hypothalamus controls the release of FSH from anterior pituitary. Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) are intimately involved in the control of the growth and reproductive activities of the gonadal tissues, which synthesize and secrete male and female sex hormones. The levels of circulating FSH and LH are controlled by these sex hormones through a negative feedback. Like LH, TSH and HCG, FSH consists of two subunits – alpha and beta, its biological and immunological properties being dependent on the hormone-specific beta subunit. In females, FSH stimulates the growth and maturation of ovarian follicles. At the beginning of normal menstrual cycle FSH level is higher that at the final stage of follicular phase. Peak FSH levels are seen in the middle of the cycle concomitantly with LH peak levels. Increased estradiol and progesterone production during luteinic phase leads to decreased FSH blood concentrations by negative feedback mechanism. The same mechanism leads to elevation of FSH levels at the end of the cycle due to decreased estrogen and progesterone concentrations, and the new cycle is initiated. In men, FSH regulates growth of seminiferous tubules and maintenance of spermatogenesis. However, androgens, unlike estrogen, do not lower FSH level, therefore demonstrating a feedback relationship only with serum LH. High levels of FSH in women are seen in menopause, preliminary ovarian failure, agenesia of ovaries; in men elevated FSH levels may be found in primary testicular failure, dysgenesia of seminiferous tubules, delayed sexual maturation, and Klinefelter syndrome. Elevated concentrations are also found in cases of starvation, renal failure, hyperthyroidism,

cirrhosis and after intake of clomifen, l-DOPA. Decreased FSH levels are found in hypopituitarism and after intake of oral contraceptives, phenotiazine, estrogens.

PRINCIPLE

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to β chain human FSH-antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Second antibodies – murine monocnoclonal to α chain human LH/FSH/HCG, labelled with peroxidase enzyme, are then added into the microwells. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

ASSAY RESTRICTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA

Immunoassay, the possibility of interference cannot be excluded.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

STORAGE CONDITIONS

The unopened kit should be stored at 2-8˚C. Immediately after use remaining reagents should be returned to cold storage at 4˚C

MATERIALS SUPPLIED IN THIS KIT

1. FSH Strip Plates, 8 X 12 wells. Qty: 1
2. Calibrators: C1: 0, C2: 5, C3: 25, C4: 50, C5: 100 IU/l, 5pcs
3. Control Serum, Qty: 1
4. Conjugate, Qty: 1
5. Substrate Solution, Qty: 1
6. Wash Buffer Concentrate 21X, Qty: 1
7. Stop Solution, Qty: 1
8. Plate Sealing Tape, Qty: 2
9. Instruction Manual, Qty: 1

PRECAUTIONS

Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer. 2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8 °C in their pouch with the desiccant

provided. 3. Mix all reagents before using. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25 °C).

TECHNICAL HINTS

• When mixing or reconstituting protein solutions, always avoid foaming.

• 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.

• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

• When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

• Substrate solution should remain colourless until added to the plate. Keep Substrate solution protected from light. Substrate solution should change from colorless to gradations of blue.

• Stop Solution should be added to the plate in the same order as the Substrate solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate solution.

SAMPLE COLLECTION & STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided. Specimens may be stored for up to 48 hours at +2…+8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/ thawing should be avoided.

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20˚C. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20˚C. Avoid repeated freeze-thaw cycles. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored at -20 ˚C. 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.

REAGENT PREPARATION

—All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18…+25 °C) before use.

—All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.

—It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.

—Prepare washing solution from the concentrate BUF WASH 21X by 21 dilutions in distilled water.

—It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

ASSAY PROCEDURE

1 Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators CAL 1–5 and control samples CONTROL and two wells for each unknown sample.

DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.

2 Dispense 100 µl of conjugate solution into the wells.

3 Pipet 50 µl of calibrators CAL 1–5, control samples CONTROL and unknown samples into the wells.

4 Incubate 60 minutes at 37 °C. Keep wells covered.

5 Prepare washing solution by 21x dilution of washing solution concentrate (BUF WASH 21X) by distilled water. Wash the strips 5 times.

6 Dispense 100 µl of SUBS TMB into the wells

7 Incubate 10–20 minutes at +18…+25 °C

8 Dispense 100 µl of STOP into the wells.

9 Measure OD (optical density) at 450 nm.

10 Set photometer blank on first calibrator

11 Apply lin-log method for data reduction.-

EXPECTED VALUES AND CHARACTERISTICS

The following normal range is recommended (see below).

NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Sex, age	Units, IU/l	
	Lower limit	Upper limit
Children under 11 yrs	-	4.0
Males	0.8	25
Females		
Menstrual cycle:		
follicular phase	3.0	12
luteinic phase	2.0	12
ovulation	6.0	25
post menopausal	10.0	150

PERFORMANCE CHARACTERISTICS

Analytical specificity / Cross reactivity

TSH<0.1, LH<0.1 and HCG<0.1 %wt/wt

Sensitivity of the assay was assessed as being 0.3 IU/l.

Linearity was checked by assaying dilution series of 5 samples with different concentrations. Linearity percentages obtained ranged within 90 to 110%.

Recovery was estimated by assaying 5 mixed samples with known concentrations. The recovery percentages ranged from 90 to 110%

CALCULATION

Calculate the mean absorbance values (OD450) for each pair of calibrators and samples. Plot a calibration curve on graph paper: OD versus FSH concentration. Determine the corresponding concentration of FSH in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

VALIDITY & STORAGE: 12 months (at 2-8℃, unopened).