INTENDED USE

The Follicle Stimulating Hormone (FSH) Test Kit is an ELISA used for the determination of FSH in serum or plasma.

ANALYTICAL PRINCIPLE

Samples along with calibrators and controls and FSH Antibody-HRP conjugate are incubated in FSH antibody coated microwells. During incubation, a sandwich complex between the FSH Antibody-HRP conjugate, FSH from samples, standards, and controls, and FSH Antibody coated on the microwells forms on the surface of the microwells. After incubation, unbound FSH Antibody-HRP conjugate is washed from the microwells. TMB is allowed to react with any FSH Antibody-HRP conjugate remaining on the microwells in the sandwich complex. The reaction is stopped and the microwells are read on a microplate reader. The level of FSH present in the samples, standards, and controls are proportional to the intensity of color that is produced.

SPECIMEN REQUIREMENTS

Serum collected in red top tube, EDTA plasma, or SST tubes are acceptable. **Do not use excessively lipemic or hemolyzed samples.** Samples are stable for up to 2 days refrigerated (2-8°C) and up to 1.5 years frozen (-15 to -35°C).

REAGENTS

Precautions & Safety Notes

- WEAR LATEX GLOVES, FACE SHIELDS AND A LAB COAT WHEN HANDLING SPECIMENS AND OTHER HAZARDOUS REAGENTS
- FOR *IN VITRO* USE, POTENTIAL BIOHAZARDOUS MATERIAL. HANDLE ASSAY REAGENTS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.
- Sample Diluent, HRP Conjugate, TMB Solution, Calibrators, Controls, and 20X Wash Buffer are preserved with < 0.1% ProClin 300. Avoid contact of these reagents with skin or eyes.

Kit Components

ID	Name	Part Number	96 QTY	480 QTY
	FSH Test Kit	**FSH-3000**	**96 tests**	**480 tests**
A	Microwell Plate	**FSH-3001**	**96**	**5 x 96**
B	HRP Conjugate	**FSH-3002**	**10 mL**	**50 mL**
C1 – C6	Set of 6 Calibrators (0, 5, 10, 25, 50, 100 mIU/mL)	**FSH-3003(A-F)**	**400 µL/vial**	**1.5 mL/vial**
D	Control Level 1	**FSH-3004A**	**500 µL/vial**	**1.5 mL/vial**
E	Control Level 2	**FSH-3004B**	**500 µL/vial**	**1.5 mL/vial**
TS	TMB Solution Keep away from light	**TMBS-1001**	**10 mL**	**50 mL**
SS	Stop Solution 1N Sulfuric Acid	**SSFEA-1001**	**10 mL**	**50 mL**
WB	20X Wash Buffer Concentrate	**WBFEA-1003**	**20 mL**	**50 mL**
	Microwell film seal			

MATERIALS

Materials needed but not supplied:

- Variable Pipettors and Tips
- 12x75 mm disposable borosilicate glass culture tubes
- Refrigerator (for kit storage)
- Vortexer
- ELISA Plate Washer
- ELISA Plate Reader
- ELISA Plate Shaker

REAGENT PREPARATION

1X Wash Buffer Preparation

1. Add 1 unit of volume of 20X Wash Buffer Concentrate (**WB**) to 19 units of volume of DI water. For example, add **20 mL** 20X Wash Buffer Concentrate (**WB**) to **380 mL** of DI or purified water.
2. Mix the Wash Buffer Preparation well.

PROCEDURE PRECAUTIONS

- Bring reagents to room temperature before use.
- Use clean instruments & equipments.
- Microwells can be snapped to select exact number of wells.
- Unused microwells should be sealed in pouch.
- Handle microwells with care.
- Minimize air bubbles in microwells.
- Microwells should not contain liquid after the washing steps. To ensure that liquids are removed from microwells after washing steps, microwells can be tapped against clean paper towels.
- It is recommended to perform calibrator, controls, and unknowns in duplicates.
- Store unused calibrator and controls at 2°C - 8°C for up to 3 weeks.
- Store unused calibrator and controls at -20°C ± 5 °C for up to 6 months.

PROCEDURE

1. Arrange the required number of microwells on the microwell plate in accordance to the Microwell Assignment Table below.
2. Dispense **50 µL** of calibrators, controls (**C1 – C6, D, E**) and unknowns to microwells (**A**).
3. Dispense **100 µL** of FSH Antibody-HRP conjugate (**B**) and unknowns to microwells (**A**).
4. Shake the wells manually for 30 seconds. Avoid splashing the contents of the microwells.
5. Cover microwells with microwell film seal.
6. Incubate at **room temperature** for **1 hour ± 5 minutes**.
7. Wash each microwell **3 times** with **300 µL** of **1X Wash Buffer Preparation**. Tap the microwells against clean paper towels to ensure that no liquid remains
8. Dispense **100 µL** of TMB Solution (**TS**) to each microwell.
9. Incubate the microwells for **15 minutes ± 1 minute** at **room temperature** and **away from light**.
10. Dispense **100 µL** of Stop Solution (**SS**) to each well.
11. Read the wells in a microwell reader at **450 nm**.

Microwell Assignment Table

Microwell	Name
A1 - A2	Calibrator 1
B1 - B2	Calibrator 2
C1 - C2	Calibrator 3
D1 - D2	Calibrator 4
E1 - E2	Calibrator 5
F1 - F2	Calibrator 6
G1 - end	unknowns

CALCULATIONS

A standard curve is used to calculate the concentration of unknowns.

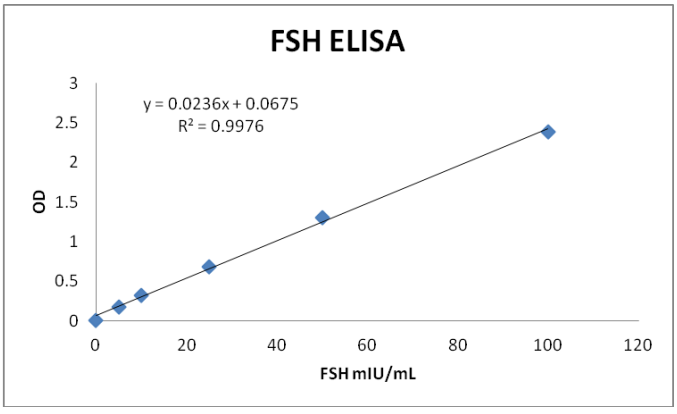
1.Use the mean OD values of the calibrators to plot a standard curve on a linear graph. Plot absorbance on y axis and concentration on x axis.

2. Plot the OD of the controls and unknowns using the standard curve to determine the concentration. Multiply the concentration found by the sample dilution factor, if a dilution was performed.

TYPICAL RESULTS

Standard Curve and Control Results			
mIU/mL	**OD 1**	**OD 2**	**Mean OD**
0	0.013	0.012	0.013
5	0.177	0.165	0.171
10	0.311	0.331	0.321
25	0.680	0.692	0.686
50	1.260	1.354	1.307
100	2.350	2.420	2.385

Standard Curve



POST PROCEDURE NOTES

- Abnormal and borderline results should be re-assayed for verification.

Manufacturer	
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