Human Follicle Stimulating Hormone (hFSH) Enzyme Immunoassay Test

Cat. No: KBBA08

Ver2.0

Immunoassay for the quantification of hFSH in human Serum

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Introduction:

Human follicle stimulating hormone (FSH) is a glycoprotein hormone produced by the anterior pituitary gland. There are three other glycoprotein hormones, namely Thyroid Stimulating Hormone, Luteinizing Hormone (both produced by anterior pituitary gland) and Human Chorionic Gonadotropin (produced by the placenta) which are structurally similar. Each hormone has an alpha and beta subunit. The α subunits of each hormone are similar while the β subunit is specific to each hormone. The α subunits contain 92 amino acids while the β subunits vary with each hormone. The β subunit of both FSH and LH contain 115 amino acids, TSH 110 amino acids, and hCG 147 amino acids. The FSH and LH hormones function differently in females and males. It is to be noted that in women the growth and maturation of the ovarian follicle is dependent on FSH, while in men both LH and FSH act on the testes.

Intended Use:

This kit is intended for determination of Human Follicle Stimulating Hormone (hFSH) in human serum.

Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. Antibodies to FSH are pre-coated onto microwells. Samples and standards are pipetted into microwells and anti-FSH antibodies linked to HRP is added to the wells. FSH present in the sample and standards will bind to the plate and HRP Conjugate simultaneously. Washing is done to remove the unbound samples, standards and HRP conjugate. The ready to use substrate solution (TMB) is added to microwells and color develops directly proportional to the amount of FSH in the standards and sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti-FSH coated Microtiter plate (96 wells) – 1 no
2. Control 1 – 0.5 ml
3. Control 2 - 0.5 ml
4. FSH Standard 0 IU/L – 2ml
5. FSH Standard (0.5 ml/vial) - 5, 10, 20, 50 and 100 IU/L
6. Anti-FSH Antibody:HRP Conjugate – 300 µl/vial
7. Assay Buffer – 25 ml
8. Wash Buffer – 50 ml
9. TMB Substrate – 16 ml
10. Stop Solution – 6 ml
11. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25µl to 1000µl
3. Deionised (DI) water
4. ELISA Plate shaker
5. Wash bottle or automated microplate washer
6. Log-Log graph paper or software for data analysis

Handling/Storage:

1. All reagents should be stored at 2^0C to 8^0C for stability.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. Before using, bring all components to Room Temperature (18-25 ºC). Upon assay completion return all components to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists
2. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
3. For Research or Manufacturing use only.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.

2. Bring all reagents to Room Temperature before use.

3. To make Wash Buffer (1X); dilute 50ml of 10X Wash Buffer in 450 ml of DI water.

4. Anti-FSH Antibody:HRP Conjugate; dilute 240µl of Anti-FSH antibody: HRP in 12ml of Assay Buffer.

Specimen Collection and Preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at room temperature (RT).

2. Pipette out 25µl of **Standards, Controls** and **Samples** into the respective wells as mentioned in the work list.

3. Pipette 100µl of **assay buffer** into each well.

4. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.

5. Aspirate and wash plate 3 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper.

6. Pipette 100µl of the **anti-FSH:HRP conjugate** into each well.

7. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.

8. Aspirate and wash plate 3 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper.

9. Pipette 100µl of **TMB substrate** into each well.

10. Incubate on a plate shaker for 10-15 minutes at room temperature.

11. Pipette 50µl of stopping solution into each well.

12. Read the plate on a microwell plate reader at 450nm within 20 minutes after addition of the stopping solution.

Example of a Work list

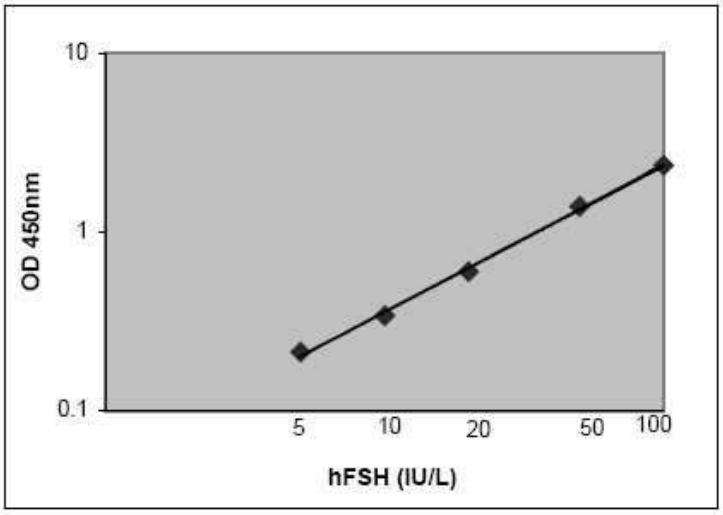
Well #	Contents	Abs at 450nm	Mean Absorbance	IU/L hFSH equiv.
1A 2A	Zero Std Zero Std			
1B 2B	5 IU/L 5 IU/L			
1C 2C	10 IU/L 10 IU/L			
1D 2D	20 IU/L 20 IU/L			
1E 2E	50 IU/L 50 IU/L			
1F 2F	100 IU/L 100 IU/L			
1G 2G	Control 1 Control 1			
1H 2H	Control 2 Control 2			

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards, Controls and Samples. Subtract the Mean Absorbance of the Zero Standards (background) from each well. Using log-log graph paper or a computer program, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit straight line through the standard points. Draw the best fit straight line through the standard points. To determine the unknown FSH concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the FSH Concentration. Computer based curve-fitting software may be preferred.
If a sample reads more than 100 IU/L then dilute it with Standard 0 at a dilution of no more than 1:8. The result should be multiplied with the dilution factor.

Typical Data:

This standard curve was generated at KRISHGEN for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

1. <u>Sensitivity:</u>

a) **Limit Of Detection:** It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be 1 IU/L.

b) **Limit of Quantitation:** It is defined as the lowest concentration for which Coefficient of Variation is <20%. The LOQ is found to be <5 IU/L.

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Troubleshooting

Problem	Possible cause	Investigation/Actions
High Absorbances	1. Cross-contamination from other specimens	> Repeat assay taking care when washing and pipetting.
	2. Insufficient or inefficient washing or reading	> Check washer efficiency
	3. Wavelength of filter not correct.	> Check that the wavelength is 450nm. If a dual wavelength spectrophotometer is available, set the reference filter between 600-650 nm.
	4. High assay background.	> Repeat assay and include a well that contains only sample diluent or sample absorbent (i.e. a blank well).
	5. Contaminated TMB	> Check that TMB is colorless or faint blue.
	6. Incubation time too long or incubation temperature too high.	> Check incubation time and temperature. > Check incubator is at the correct temperature.
	7. Incorrect dilution of serum	> Repeat assay, ensuring correct serum dilution is used.
Low Absorbances	1. Incubation time too shot or incubation temperature too low.	> Ensure time and temperature of assay incubation are correct
	2. Incorrect dilution or pipetting of sera	> Check incubator is set at the correct temperature.
	3. Incorrect filter wavelength.	> Repeat assay ensuring correct dilutions and volumes are used
	4. Contaminated Conjugate solution.	> Ensure controls are sufficiently mixed. > Check the wavelength is set at 450nm. If a dual wavelength spectrophotometer is available, set the reference filter between 600-650nm. > Dispense conjugate directly from the bottle using clean pipette tip; avoid transferring Conjugate to another container if possible. > Do not return unused Conjugate to bottle. > Ensure all pipettes and probes used to dispense the Conjugates are clean and free from serum, detergent and bleach.
	5. Kit has expired.	> Check expiration date of kit and do not use if expired.
	6. Air blank reading high	> Investigate causes of high background absorbance.
	7. Incorrect storage of kit.	> Ensure kit is stored at 2-8ºC, plate is sealed in foil pouch and desiccant sachet is blue/purple.
	8. Kit reagents not equilibrated at room temperature	> Allow sufficient time for reagents to equilibrate to room temperature prior to assay.
	9. Incorrect reagents used.	> Check the reagents used match those listed on the specification sheet.
	10. Over washing of plate (e.g. inclusion of a long soak step).	> Repeat assay using recommended wash procedure.
Poor Duplicates	1. Poor mixing of samples.	> Mix reagents gently and equilibrate to room temperature.
	2. Poor pipette precision	> Calibration may need to be checked. > Check pupating technique-change pipette tip for each sample and ensure excess liquid is wiped from the outside of the tip.
	3. Addition of reagents at inconstant timing intervals; reagent addition takes too long, air bubbles when adding reagents.	> Use consistent timing when adding reagents. > Ensure all dilutions are made before commencing addition to plate. > Improve pipetting technique and skill.
	4. Inefficient washing - Wash buffer left in wells, inconsistent washing, inadequate washing.	> Tap out wash buffer after washing. > Check wells are sufficiently and uniformly filled and aspirated when washing.
	5. Reader not calibrated or warmed up prior to plate reading.	> Check reader precision > Check reader manual to ascertain warm up time of instrument.
	6. Optical pathway not clean	> Gently wipe bottom of plate. > Check reader light source and detector are clean.
	7. Spillage of liquid from wells	> Repeat assay, taking care not to knock the plate or splash liquid
	8. Serum samples exhibit microbial growth, haemolysis or lipaemia.	> It is not recommended to use serum samples exhibiting microbial
	9. Uneven well volumes due to evaporation.	> Cover plate with a lid or plate sealer (not provided).

All wells Yellow	1. Contaminated TMB.	>	Check TMB is colorless or faint blue.
	2. Contaminated reagents (e.g. Conjugate, Wash buffer	>	Check reagents for turbidity.
	3. Incorrect dilution of serum.	>	Repeat assay, ensuring correct serum dilution is used.
	4. Incorrect storage of kit.	>	Ensure kit is stored at 2-8ºC, plate is sealed in foil pouch and desiccant sachet is blue / purple.
	5. Inefficient washing- Wash buffer left in wells, inconsistent washing, inadequate washing.	>	Tap out wash buffer after washing.
		>	Check wells are sufficiently and uniformly filled an aspirated washing.
	6. If conjugate reconstitution is required- Conjugate reconstituted incorrectly.	>	Repeat assay ensuring Conjugate is reconstituted according to assay method.
All wells Negative			
	1. Test not performed correctly – correct reagents not added or not added in the correct sequence	>	Check procedure and check for unused reagents.
		>	Ensure that Stop Solution was not added before Conjugate or TMB.
		>	Ensure that serum was diluted in correct Sample diluent; e.g. do not use Sample Absorbent for an IgG ELISA.
	2. Contaminated Conjugate solution.	>	Dispense Conjugate directly from the bottle using a clean pipette tip; avoid transferring Conjugate to another container if possible.
		>	Do not return unused Conjugate to bottle.
		>	Ensure all pipettes and probes used to dispense the Conjugate are clean and free from serum, detergent and bleach.
	3. Over- washing of plate (e.g. inclusion of a long soak step).	>	Repeat assay using recommended wash procedure.
	4. Incorrect storage of kit.	>	Ensure kit is stored at 2-8ºC, plate is sealed in foil pouch and desiccant sachet is blue / purple.
	5. Wash Buffer made up with Stop Solution instead of Wash Buffer Concentrate	>	Ensure Wash Buffer is made up correctly.

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