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ab108678

Follicle Stimulating Hormone Human ELISA Kit

For the quantitative measurement of Human Follicle Stimulating Hormone (FSH) concentration in serum.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

ab108678 Follicle Stimulating Hormone Human ELISA Kit is a immunoenzymatic colorimetric method for quantitative determination of Follicle Stimulating Hormone in serum.

Follicle Stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,500 daltons. The a-subunit is similar to other pituitary hormones [luteinizing stimulating hormone (LH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (hCG)] while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. Stimulation by gonadotropin-releasing hormone (GnRH) causes release of FSH, as well as LH, from the pituitary and is transported by the blood to their sites of action, the testes or ovary. In men, FSH acts on the Sertoli cells of the testis, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly supports spermatogenesis. In women, FSH acts on the granulosa cells of the ovary, stimulating steroidogensis. All ovulatory menstrual cycles have a characteristic pattern of FSH, as well as LH, secretion.

2. Protocol Summary

In the ab108678 method, calibrators, specimens and/or controls (containing the native FSH antigen) are first added to streptavidin coated wells.

Biotinylated monoclonal and horseradish peroxidase (HRP) labeled antibodies are added and the reactants are mixed. The different types of antibodies used have high affinity and specificity and are directed against distinct and different epitopes of FSH. Reaction between the various FSH antibodies and native FSH occurs in the microwells without competition or steric hindrance, forming a soluble sandwich complex.

Simultaneously, the complex is fixed to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by aspiration.

The activity of the conjugated HRP is quantified by reaction with TMB substrate to produce blue color. The reaction is terminated by adding stop solution which turns the blue color into yellow. The absorbance is measured on a plate reader.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt, Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-FSH conjugate	1x12 mL	4°C
Coated Wells	12 x 8 wells	4°C
Foil Cover	1	4°C
FSH Control	1x1 mL	4°C
Stop Solution	1x15 mL	4°C
Strip holder	1	4°C
TMB Substrate Solution	1x15 mL	4°C
10X Wash solution	1x50 mL	4°C
FSH Standard 0 (0 mIU/ml)	1x1 mL	4°C
FSH standard 1 (5 mIU/ml)	1x1 mL	4°C
FSH Standard 2 (10 mIU/ml)	1x1 mL	4°C
FSH Standard 3 (25 mIU/ml)	1x1 mL	4°C
FSH Standard 4 (50 mIU/m)	1x1 mL	4°C
FSH Standard 5 (100 mIU/ml)	1x1 mL	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 50 µl (precision better than 1.5%)
- Dispenser for repetitive deliveries of 100µl and 300 µl volumes with a precision better than 1.5%
- Vortex tube mixer
- Distilled water
- Quality control material
- Timer

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Coated snap-off Strips

The ready to use break apart snap-off strips are coated with Streptavidin. Store at 2-8°C. Open the bag only when it is at room temperature. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2-8°C. Do not remove the adhesive sheets on the unused strips.*

9.2 Anti-FSH Conjugate

The bottle contains 13 ml of a ready-to-use conjugate mixture of HRP-labeled polyclonal anti-FSH antibodies and biotinylated monoclonal anti-FSH antibodies.

9.3 FSH Standards

Each of the 6 vials contains 1 ml standard solution of the concentration mentioned in Kit Contents. The standards are ready to use.

9.4 TMB Substrate Solution

The bottle contains 12 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2-8°C in the dark. *The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first use the TMB substrate solution is still stabile for another 6 months if stored at 2-8 °C.*

9.5 Stop Solution

The bottle contains 12 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2-8°C. After first use stable until expiry date.

9.6 Wash Solution

Dilute the 10x concentrated wash solution to 500 ml with distilled or deionised water in a suitable storage container.

10. Standard Preparation

The standards come ready to use and are calibrated against the (WHO 2nd IRP 78/549) and have approx. the following concentrations:

Standard 0: 0 mIU/ml

Standard 1: 5 mIU/ml

Standard 2: 10 mIU/ml

Standard 3: 25 mIU/ml

Standard 4: 50 mIU/ml

Standard 5: 100 mIU/ml

11. Sample Preparation

Use human serum as samples with this assay. The blood should be collected in a venipuncture tube without additives or anticoagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of 5 days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing.

For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

Precaution:

- The reagents contain Proclin 300® as preservative
- Do not use heavily haemolysed or highly lipemic samples.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- This method allows the determination of FSH from 0.6 to 100 mIU/ml.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Please allocate at least:

1 well	(e.g. A1)	for the substrate blank
2 wells	(e.g. B1+C1)	for standard 0
2 wells	(e.g. D1+E1)	for standard 1
2 wells	(e.g. F1+G1)	for standard 2
2 wells	(e.g. H1+A2)	for standard 3
2 wells	(e.g. B2+C2)	for standard 4
2 wells	(e.g. D2+E2)	for standard 5
2 wells	(e.g. F2+G2)	for control

12.1 Dispense 50 µl standards and samples into their respective wells. Add 100 µl conjugate to each well. Leave well A1 for substrate blank.

12.2 Cover wells with the foil supplied in the kit.

12.3 Incubate for 1 hour at room temperature (22 – 28 °C).

12.4 When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values

12.5 Dispense 100 µl TMB Substrate Solution into all wells.

12.6 Incubate for exactly 15 min at room temperature (22 – 28°C) in the dark.

12.7 Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
Any blue color developed during the incubation turns into yellow.

12.8 Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.

12.9 Analyze the data as described below.

12.9.1 Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.
If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

12.9.2 Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and sample.

12.9.3 Where applicable calculate the mean absorbance values of all duplicates.

12.10 Quality control:

Each laboratory should assay controls at normal, high and low levels range of FSH for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.
If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

12.12 Calculation

12.12.1 Automated method

Use the 4 parameter logistic (preferred) or the smoothed cubic spline function as calculation algorithm.

12.12.2 Manual method

A dose response curve is used to ascertain the concentration of follicle stimulating hormone in unknown specimens.

12.12.2.1 Record the OD obtained from the printout of the microplate reader as outlined in Example 1

12.12.2.2 Plot the OD for each duplicate calibrator versus the corresponding FSH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

12.12.2.3 Draw the best-fit curve through the plotted points

12.12.2.4 To determine the concentration of FSH for an unknown, locate the average OD of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged).

NOTE: The data presented in Example 1 are for illustration only and should not be used in lieu of a standard curve prepared with each assay. Assigned values for standards are lot specific.

13. Typical Data

Example 1				
Sample ID	Well number	OD	Mean OD	Value mIU/ml
Standard 0	A1	0.092	0.072	0
	B1	0.053		
Standard 1	C1	0.375	0.371	5
	D1	0.367		
Standard 2	E1	0.573	0.581	10
	F1	0.588		
Standard 3	G1	1.412	1.301	25
	H1	1.190		
Standard 4	A2	1.923	1.944	50
	B2	1.965		
Standard 5	C2	2.739	2.694	100
	D2	2.650		
Control	E2	0.714	0.697	13.89
	F2	0.6881		
Sample	G2	1.671	1.627	43.8
	H2	1.496		

14. Typical Sample Values

SENSITIVITY –

The lowest detectable concentration of FSH by this assay is 0.6 mIU/ml.

PRECISION –

Intra-Assay: Within-run precision was determined by replicate determinations of three different control sera in one assay. The within assay variability is shown.

Serum Sample	1	2	3
Number of Replicates	20	20	20
Mean FSH (mIU/ml)	5.4	15.6	37.9
Standard Deviation	0.46	1.32	3.56
Coefficient of Variation (%)	9.6	8.5	9.4

Inter-Assay: Between-run precision was determined by replicate measurements of three different control sera in several different assays. The between assay variability is shown below.

Serum Sample	1	2	3
Number of Replicates	10	20	20
Mean FSH (mIU/ml)	5.9	16.9	35.3
Standard Deviation	0.66	1.59	4.16
Coefficient of Variation (%)	11.2	9.4	11.8

15. Assay Specificity

The cross-reactivity of ab108678 Follicle Stimulating Hormone Human ELISA Kit to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Follicle Stimulating Hormone needed to produce the same OD.

Material Tested	Cross Reactivity	Concentration
Follitropin (FSH)	1.0000	---
Lutropin Hormone (hLH)	<0.0001	1000ng/ml
Chorionic Gonadotropin (hCG)	<0.0001	1000ng/ml
Tireotropin (TSH)	<0.0001	1000ng/ml

Please contact our Technical Support team for more information.

16. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)

	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4 °C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended

	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

17. Notes

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