FSH ELISA

OKDA00027

For Research Use Only

Aviva Systems Biology, 5754 Pacific Center Blvd., Suite 201, San Diego, CA 92121, Tel: 1-858-552-6979

1 INTRODUCTION

1.1 Intended Use

The **Aviva FSH ELISA** is an enzyme immunoassay for the quantitative measurement of Follicle Stimulating Hormone (FSH) in serum.

1.2 Summary and Explanation

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 sex hormones through a negative feedback relationship (1,2).

FSH is a glycoprotein secreted by the basophil cells of the anterior pituitary. Gonadotropin-releasing hormone (GnRH), produced in the hypothalamus, controls the release of FSH from the anterior pituitary. Like other glycoproteins, such as LH, TSH, and HCG, FSH consists of subunits designated as alpha and beta. Hormones of this type have alpha subunits that are very similar structurally, therefore the biological and immunological properties of each are dependent on the unique beta subunit (3,4,5).

In the female, FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells; follicular steroidogenesis is promoted and LH production is stimulated. The LH produced then binds to the theca cells and stimulates steroidogenesis. Increased intraovarian estradiol production occurs as follicular maturation advances, thereupon stimulating increased FSH receptor activity and FSH follicular binding. FSH, LH, and estradiol are therefore intimately related in supporting ovarian recruitment and maturation (6,7,9).

FSH levels are elevated after menopause, castration, and in premature ovarian failure. The levels of FSH may be normalized through the administration of estrogens, which demonstrate a negative feedback mechanism. Abnormal relationships between FSH and LH, between FSH and estrogen have been linked to anorexia nervosa and polycystic ovarian disease. Although there are significant exceptions ovarian failure is indicated when random FSH concentrations exceed 40 mIU/mL (8).

The growth of the seminiferous tubules and maintenance of spermatogenesis are regulated by FSH. However, androgens, unlike estrogens, do not lower FSH levels, therefore demonstrating a feedback relationship only with serum LH (10,11,12). For reasons not fully understood, azospermic and oligospermic males usually have elevated FSH levels. Tumors of the testes generally depress serum FSH concentrations, but levels of LH are elevated, as determined by radioimmunoassay. It has been postulated that the apparent LH increase may be caused by crossreactivity with hCG-like substances secreted by tumors of the testes (11,12). 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 (1,3).

2 PRINCIPLE OF TEST

The Aviva FSH ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a FSH molecule.

An aliquot of sample containing endogenous FSH is incubated in the coated well with enzyme conjugate, which is an anti-FSH monoclonal antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of FSH in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of FSH in the sample.

3 WARNINGS AND PRECAUTIONS

- This kit is for professional use only.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for hiv i/ii, hbsag and hcv by fda approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °c to 8 °c in the sealed foil pouch and used in the frame provided.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21-26°c) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.

- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Avoid contact with *stop solution* containing 0.5 m h_2so_4. It may cause skin irritation and burns.
- Some reagents contain proclin, bnd and mit as preservatives. In case of contact with eyes or skin, flush immediately with water.
- Tmb substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- For information on hazardous substances included in the kit please refer to material safety data sheets. Material safety data sheets for this product are available upon request directly from DIAsource.

4 REAGENTS

4.1 Reagents provided

1. **Microtiterwells,** 12 x 8 (break apart) strips, 96 wells.
   Wells coated with anti-FSH monoclonal antibody.

2. CAL | N
   FSH Calibrators. N= 0 to 5, 6 vials (lyophilized), 1 mL
   Concentration : 0,5; 10; 20; 50; 100 mIU/mL
   Conversion : 6 mIU/mL
   The calibrators are calibrated against 1. International Calibrator for Follicle Stimulation Hormone (FSH), human recombinant for immunoassay NIBSC code 92/510
   See “Preparation of Reagents”
   Contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.

3. Ab | HRP
   Enzyme Conjugate, 1 vial, 11 mL. Ready for use.
   Anti-FSH antibody conjugated to horseradish peroxidase.
   Contains 0.03 % Proclin 300, 0.015 % BND and 0.010 % Mit as preservative.

4. CHROM | TMB
   Substrate Solution, 1 vial, 14 mL. Ready for use.
   Tetramethylbenzidine (TMB)

5. STOP | SOLN
   Stop Solution, 1 vial, 14 mL. Ready for use.
   Contains 0.5 M H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.

   BND = 5-bromo-5-nitro-1,3-dioxane
   MIT = 2-methyl-2H-isothiazol-3-one

Note: Additional *Calibrator 0* for sample dilution is available on request.

4.2 Material required but not provided

- A microtiter plate calibrated reader (450±10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.

4.3 Storage Conditions

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above.

4.4 Reagents Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Calibrators
Reconstitute the lyophilized contents of the calibrator vial with 1 mL Aqua dest.
Note: *The reconstituted calibrators are stable for 2 months at 2-8°C. For longer storage freeze at -20°C.*

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, DIAsource has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Only serum should be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples receiving anticoagulant therapy may require increased clotting time.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying.
Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest calibrator, the specimens can be diluted with *Ctandard 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) dilution 1:10: 10 µL Serum + 90 µL Calibrator 0 (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Calibrator 0 (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each calibrator, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Pipetting of all calibrators, samples, and controls should be completed within 6 minutes. (Note this especially for manual pipetting.)

6.2 Test Procedure

Each run must include a calibration curve.

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **25 µL** of each *Calibrator, controls* and samples with new disposable tips into appropriate wells.
3. Dispense **100 µL** *Enzyme Conjugate* into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **30 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with aqua dest (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL** of *Substrate Solution* to each well.
7. Incubate for **10 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of *Stop Solution* to each well.
9. Determine the absorbance (OD) of each well at **450±10 nm** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of calibrators, controls and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each calibrator against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the calibration curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this calibration curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Calibration Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Calibrator	Optical Units (450 nm)
Calibrator 0 (0 mIU/mL)	0.07
Calibrator 1 (5 mIU/mL)	0.16
Calibrator 2 (10 mIU/mL)	0.26
Calibrator 3 (20 mIU/mL)	0.44
Calibrator 4 (50 mIU/mL)	0.92
Calibrator 5 (100 mIU/mL)	1.71

7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy specimen, using the Aviva FSH ELISA the following values are observed:

Population	5% - 95% Percentile [mIU/mL]
Sample M	2.0 – 10.0
Sample F	
Follicular Phase	2.0 - 10.0
Mid-cycle	7.0 - 20.0
Luteal Phase	2.0 - 10.0
Post-Menopausal	20.0 –100.0

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DIAsource directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.86 – 100 mIU/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Hormone Tested	Concentration	Produced Color Intensity Equivalent to FSH in serum (mIU/mL)
hCG (WHO 1st IRP75/537)	10.000 mIU/mL 50.000 mIU/mL 100.000 mIU/mL	0 0 0
TSH (WHO 2nd IRP 80/558)	50 µIU/mL 100 µIU/mL	0 0
LH (WHO 1st IRP 68/40)	100 mIU/mL 250 mIU/mL 500 mIU/mL	0 0 0
Prolactin (WHO 1st IRP 75/504)	100 ng/mL 200 ng/mL	0 0
hGH (WHO 1st IRP 66/217)	100 ng/mL 200 ng/mL	0 0

9.3 Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of *Calibrator 0* and was found to be 0.856 mIU/mL.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	1	2	3
Mean (mIU/mL)	7.37	14.24	38.13
SD (mIU/mL)	0.58	0.64	1.60
CV (%)	7.91	4.50	4.18
n =	10	10	10

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	1	2	3
Mean (mIU/mL)	7.33	13.85	37.42
SD (mIU/mL)	0.53	0.81	1.93
CV (%)	7.18	5.84	5.15
n =	11	11	11

9.5 Recovery

Samples have been spiked by adding FSH solutions with known concentrations in a 1:1 ratio.
The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Endogenous FSH (mIU/mL)	Added FSH (mIU/mL)	Measured Conc. FSH (mIU/mL)	Expected * FSH (mIU/mL)	Recovery (%)
1	54.64	0	54.6		
		50	74.8	77.3	96.8
		25	56.8	52.3	108.6
		10	40.8	37.3	109.4
		5	36.2	32.3	112.1
2	24.11	0	24.1		
		50	61.7	62.1	99.4
		25	38.2	37.1	103.1
		10	24.5	22.1	110.9
		5	18.8	17.1	110.4
3	7.01	0	7.0		
		50	52.9	53.5	98.9
		25	27.0	28.5	94.6
		10	11.9	13.5	88.5
		5	7.9	8.5	93.0

(* Endogenous FSH / 2 + added FSH because of a 1:1 dilution of serum with spike material.)

9.6 Linearity

Sample	Dilution	Measured Conc. (mIU/mL)	Expected Conc. (mIU/mL)	Recovery (%)
	None	54.6	54.6	
	1:2	26.1	27.3	95.5
1	1:4	12.6	13.7	92.0
	1:8	6.1	6.8	88.8
	1:16	3.3	3.4	95.6
	None	24.1	24.1	
	1:2	13.4	12.1	111.0
2	1:4	6.4	6.0	106.0
	1:8	3.2	3.0	107.6
	1:16	1.7	1.5	111.0
	None	71.4	71.4	
	1:2	38.9	35.7	109.0
3	1:4	19.4	17.8	108.5
	1:8	9.5	8.9	106.7
	1:16	4.7	4.5	106.1

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of FSH in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 1600 mIU/mL of FSH.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national calibrators and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DIAsource.

11.2 Consequences

Consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total picture.
Only in cases where the laboratory results are in acceptable agreement with the overall picture of the sample should consequences be derived.
The test result itself should never be the sole determinant for deriving any consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES

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	Used symbols	**Symboles utilisés**
	Consult instructions for use	Consulter les instructions d'utilisation
	Storage temperature	Température de conservation
	Use by	Utiliser jusque
LOT	Batch code	Numéro de lot
REF	Catalogue number	Référence de catalogue
CONTROL	Control	Contrôle
IVD	In vitro diagnostic medical device	Dispositif médical de diagnostic in vitro
	Manufacturer	Fabricant
	Contains sufficient for <n> tests	Contenu suffisant pour <n> tests
WASH SOLN CONC	Wash solution concentrated	Solution de lavage concentrée
CAL 0	Zero calibrator	Calibrateur zéro
CAL N	Calibrator #	Calibrateur #
CONTROL N	Control #	Contrôle #
Ag 125I	Tracer	Traceur
Ab 125I	Tracer	Traceur
Ag 125I CONC	Tracer concentrated	Traceur concentré
Ab 125I CONC	Tracer concentrated	Traceur concentré
	Tubes	Tubes
INC BUF	Incubation buffer	Tampon d'incubation
ACETONITRILE	Acetonitrile	Acétonitrile
SERUM	Serum	Sérum
DIL SPE	Specimen diluent	Diluant du spécimen
DIL BUF	Dilution buffer	Tampon de dilution
ANTISERUM	Antiserum	Antisérum
IMMUNOADSORBENT	Immunoadsorbent	Immunoadsorbant
DIL CAL	Calibrator diluent	Diluant de calibrateur
REC SOLN	Reconstitution solution	Solution de reconstitution
PEG	Polyethylene glycol	Glycol Polyéthylène
EXTR SOLN	Extraction solution	Solution d'extraction
ELU SOLN	Elution solution	Solution d'elution
GEL	Bond Elut Silica cartridges	Cartouches Bond Elut Silica
PRE SOLN	Pre-treatment solution	Solution de pré-traitement
NEUTR SOLN	Neutralization solution	Solution de neutralisation
TRACEUR BUF	Tracer buffer	Tampon traceur
	Microtiterplate	Microplaque de titration
Ab HRP	HRP Conjugate	HRP Conjugué
Ag HRP	HRP Conjugate	HRP Conjugué
Ab HRP CONC	HRP Conjugate concentrate	HRP Conjugué concentré
Ag HRP CONC	HRP Conjugate concentrate	HRP Conjugué concentré
CONJ BUF	Conjugate buffer	Tampon conjugué
CHROM TMB CONC	Chromogenic TMB concentrate	Chromogène TMB concentré
CHROM TMB	Chromogenic TMB solution	Solution chromogène TMB
SUB BUF	Substrate buffer	Tampon substrat
STOP SOLN	Stop solution	Solution d'arrêt
INC SER	Incubation serum	Sérum d'incubation
BUF	Buffer	Tampon
Ab AP	AP Conjugate	AP Conjugué
SUB PNPP	Substrate PNPP	Tampon PNPP
BIOT CONJ CONC	Biotin conjugate concentrate	Biotine conjugué concentré
AVID HRP CONC	Avidine HRP concentrate	Avidine HRP concentré
ASS BUF	Assay buffer	Tampon de test
Ab BIOT	Biotin conjugate	Biotine conjugué
Ab	Specific Antibody	Anticorps spécifique
SAV HRP CONC	Streptavidin HRP concentrate	Concentré streptavidine HRP
NSB	Non-specific binding	Liant non spécifique
2nd Ab	2nd Antibody	Second anticorps
ACID BUF	Acidification Buffer	Tampon d'acidification