Atlas Medical CE

FSH ELISA

Microplate based ELISA (enzyme linked immunosorbent assay) for the quantitative determination of FSH (Follicle-stimulating Hormone) in human serum

IVD For *in vitro* diagnostic and professional use only

2°C 8°C

Store at 2-8 °C

Σ **96 Tests**

Intended use

Atlas FSH ELISA is an Immunoassay for the in *vitro* quantitative determination of follicle-stimulating hormone in human serum.

Introduction

FSH (follicle stimulating hormone), together with LH (luteinizing hormone), belongs to the gonadotropin family. FSH and LH regulate and stimulate the growth and function of the gonads(ovaries and testes) synergistically. Like LH, TSH and hCG, FSH is a glycoprotein consisting of two subunits (α- and β-chains). Its molecular weight is approx. 32000 daltons. In women, the gonadotropins act within the hypothalamus-pituitary-ovary regulating circuit to control the menstrual cycle.

FSH and LH are released in pulses from the gonadotropic cells of the anterior pituitary. The levels of the circulating hormone sare controlled by steroid hormones via negative feedback to the hypothalamus. In the ovaries FSH, together with LH, stimulates the growth and maturation of the follicle and hence also the biosynthesis of estrogens in the follicles. The FSH level shows apeak at mid-cycle, although this is less marked than with LH.Due to changes in ovarian function and reduced estrogen secretion, high FSH concentrations occur during menopause.In men, FSH serves to induce spermatogonium development. Determination of the FSH concentration is used in the elucidation of dysfunctions within the hypothalamus-pituitary gonads system. The determination of FSH in conjunction with LH is utilized for the following indications: congenital diseases with chromosome aberrations, poly cystic ovaries (PCO), amenorrhea (causes), and menopausal syndrome. Depressed gonadotropin levels in men occur in azoospermia.

Test Principle

Sandwich principle. Total duration of assay: 80 minutes.

- Sample, Anti-FSH coated microwells and enzyme labeled Anti-FSH are combined.
- During the incubation, FSH presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies.
- After washing, a complex is generated between the solid phase, the FSH within the sample and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The absorbance is proportional to the amount of FSH in the sample.

Materials

Materials provided

1. Coated Microplate
   8x12 strips, 96 wells, pre-coated with mouse monoclonal Anti-FSH.
2. Calibrator
   Six white cap vials, 1 ml each, ready to use.

Calibrator	FSH Concentration (mIU/ml)
A	0
B	5
C	20
D	50
E	100
F	200

3. Enzyme Conjugate
   One red cap vial, 11 ml of HRP (horse radish peroxidase) labeled mouse monoclonal Anti-FSH in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1%ProClin300® preservative.
4. Substrate Solution
   One brown cap vial, 11ml, ready to use, (tetramethylbenzidine)TMB.
5. Stop Solution
   One yellow cap vial, 6.0 ml of 1 mol/l sulfuric acid.
6. Wash Solution Concentrate
   One transparent cap bottle, 25 ml (40Xconcentrated), PBS-Tween wash solution.
7. Package insert.
8. 1 piece of plate cover.

Materials Required but not Provided

1. Microplate reader with 450nm and 620nm wave length absorbent capability.
2. Microplate washer.
3. Incubator.
4. Plate shaker.
5. Micropipettes and multichannel micropipettes delivering 50µlwith a precision of better than 1.5%.
6. Absorbent paper.
7. Distilled water

Warnings and Precautions

1. For *in vitro* diagnostic use only. For professional use only.
2. All products that contain human serum or plasma have been found to be non-reactive for HBsAg, HCV and HIVI/II.But all products should be reared as potential biohazards in use and for disposal.
3. Conduct the assay away from bad ambient conditions. E.g.ambient air containing high concentration of corrosive gas such as Sodium hypochlorite acid, alkaline, acetaldehyde and so on,or containing dust.
4. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
5. Do not use reagents beyond the labeled expiry date.
6. Do not mix or use components from kits with different batch codes.
7. If more than one plate is used, it is recommended to repeat the calibration curve.
8. The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

Storage

1. Store at 2-8℃.
2. Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8 °C.
3. Seal and return all unused reagents to 2-8 °C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation

1. Collect serum samples in accordance with correct medical practices.
2. Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 7 days at 2-8 °C, and 1 month at -20 °C. Freeze only once.
3. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
4. Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement.

5. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
8. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
9. Mix all reagents through gently inverting prior to use.
10. Adjust the incubator to 37 °C.
11. Prepare wash solution concentrate before measurement Stable for 2 months at ambient temperature.
12. Do not use Substrate if it appears blue.
13. Do not use reagents that are contaminated or have bacterial growth.

Quality Control

Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. Controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Wash Solution Preparation

Add deionized water to the 40X concentrated Wash Solution Concentrate. Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Procedure

1. Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
2. Add 25 µL of calibrators or samples to each well.
3. Add 100 µL of enzyme conjugate to each well.
4. Shake the microplate gently for 30 seconds to mix. Avoid bubble formation
5. Cover the plate with a plate lid and incubate at 37 °C for 60 minutes.
6. Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 350 µL of wash solution, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of 5 washes . An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
8. Add 100 µL of substrate to each well.
9. Incubate at ambient temperature (18-25℃) in the dark for reaction for 20 minutes. Do not shake the plate after substrate addition.
10. Add 50 µL of stop solution to each well.
11. Shake for 15-20 seconds to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely, and to eliminate any bubbles.
12. Read the absorbance of each well at 450 nm (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within 30 minutes of adding the stop solution.

Calculation of Results

1. Record the absorbance obtained from the printout of the microplate reader.
2. Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
3. Plot the common logarithm of absorbance against concentration in mIU/mL for each calibrator.
4. Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

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

Sample	Value (mIU/mL)	Absorbance
Calibrator A	0	0.017
Calibrator B	5	0.172
Calibrator C	20	0.545
Calibrator D	50	1.178
Calibrator E	100	2.139
Calibrator F	200	3.114
Control 1	15.38	0.43
Control 2	58.95	1.35
Sample	26.87	0.69

Limitations

1. Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
2. *in vitro* tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found. In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
3. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Performance Characteristics

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Atlas reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability*		Intermediate precision	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human Serum 1	7.41	0.608	8.21	0.656	8.85
Human Serum 2	12.93	0.821	6.35	0.790	6.11
Human Serum 3	82.31	3.523	4.28	4.165	5.06
PC Universal 1	10.38	0.738	7.11	0.679	6.54
PC Universal 2	43.21	2.277	5.27	2.251	5.21

*Repeatability = within-run precision

Method comparison

A comparison of the Atlas FSH assay (y) with the Roche Cobas FSH (x) using clinical samples gave the following correlations:
Number of samples measured: 128
Linear regression
y = 0.9874x + 0.083
r = 0.9855
The sample concentrations were between approx. 0 and 187 mIU/mL

Limits and ranges

Measuring range

0.200-200 mIU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as <0.200 mIU/mL. Values above the measuring range are reported as >200 mIU/mL.

Lower limits of measurement

Lower detection limit

Lower detection limit: 0.200 mIU/mL

The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Not necessary due to the broad measuring range.

Functional Sensitivity

0.210 mIU/mL

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Interferences

1. The assay is unaffected by icterus (bilirubin <1094 µ mol/L or<64 mg/dL), hemolysis (Hb <0.621 mmol/L or <1.0 g/dL),lipemia (Intralipid <1900 mg/dL) and biotin (<246 nmol/L or <60 ng/mL).
2. No interference was observed from rheumatoid factors up to a concentration of 2250 IU/mL.

Recovery

Criterion: Recovery within ± 10 % of initial value.

Expected values

Men:

1.1 - 14.4 mIU/mL

Women:

- Follicular phase: 3.2 - 12.7 mIU/mL.
- Ovulation phase: 4.5 - 22.8 mIU/mL.
- Luteal phase: 1.2 - 8.9 mIU/mL.
- Post menopause: 22 - 146 mIU/mL.

Studies with the Atlas FSH assay have revealed thefollowingFSH values:

Test subjects	N	FSH (mIU/mL) Percentile		
		50th	5th	95th
Men	404	4.8	1.5	13
Women				
Follicular phase	339	7	4.1	11.1
Ovulation phase	145	12.5	5.1	21.5
Luteal phase	416	3.3	1.5	8
Post menopause	209	71.4	25	135

LH/FSH quotient: Quotients have been calculated from the results obtained with the Atlas LH assay and the Atlas FSH assay in the samples of healthy women of child-bearing age. The following medians have been calculated:
Follicular phase: 0.89 (n= 331)
Luteal phase: 1.15 (n= 306)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Analytical specificity

For the monoclonal antibodies used, the following cross reactivities were found:
LH 0.045%, TSH 0.01%; hGH and hCG no cross-reactivity.

Hook effect

There is no high-dose hook effect at FSH concentration up to 2000 mIU/mL.

Literature References

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry