FSH (Follicle Stimulating Hormone) Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAFSH1	FSH Elisa	96 Tests

Intended Use:

FSH Elisa is intended to be used for the quantitative determination of FSH in Human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

FSH and LH are intimately involved in the control of the growth and reproductive activities of the gonadal tissues, which synthesize and secrete male and female sex hormones. The levels of circulating FSH and LH are controlled by these sex hormones through a negative feedback relationship. FSH is a glycoprotein that is secreted by the basophilic cells of the anterior pituitary. Gonadotropin release hormone produced in the hypothalamus, controls the release of FSH from 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 identical and therefore the biological and immunological properties depend upon the beta subunit. FSh levels are elevated after menopause, castration and in premature ovarian failure. The levels FSH may be normalized through the administration of oestrogen, which demonstrates a negative feedback mechanism. Although there are significant exceptions, ovarian failure is indicated when random FSH concentrations exceed 40 mIU/ml. The growth of saeminiferous tubules and maintenance of spermatogenesis in men are regulated by FSH. However, androgens unlike oestrogens do not lower FSH levels therefore demonstrating a feedback relationship only with serum LH. For reasons not fully understood, Azospermic and oligozpermic males usually have elecated FSH concentrations. 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.

The FSH Elisa is a sandwich principle. The assay system utilizes one anti-FSH antibody for solid phase immobilization and another mouse monoclonal antiFSH antibody in the antibody enzyme conjugate solution. The test sample is allowed to react simultaneously with the antibodies resulting in the FSH molecules being sandwiched between the solid phase and enzyme linked antibodies. After a 60 minute incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes resulting in the development of a blue colour. The colour development is stopped with the addition of 2N HCl, and the colour is changed to yellow and measured at 450nm. The concentration of FSH is directly proportional to the intensity of colour developed.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with monoclonal anti FSH antibodies. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 2 months at 2-8°C.
FSH Calibrators	6x1ml	6 vials containing FSH at concentrations of 0.0, 5, 20, 50, 100 and 200 mIU/ml made up in a human serum matrix. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. Referenced against the WHO 2nd IRP, HMG) CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable for 1 month at 2-8°C.
Enzymatic Conjugate	1x11ml	1 vial containing 11ml of HRP labelled monoclonal Anti FSH antibodies in Buffered saline. Once open, stable for 2 months at 2-8°C.
Wash Buffer Concentrate (50X)	1x25ml	PBS-Tween at pH 7.4. 50X concentrate. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x11ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable for 2 months at 2-8°C.
Stop Solution	1x6ml	Diluted Sulfuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2-8°C.

IFU, Plate lid.

Materials required but not provided:

Distilled water, Micropipettes, Incubator, Microplate Reader and Microplate washer, Absorbent paper.

Specimen Collection:

- Collect serum samples in accordance with correct medical practices.
- 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. Recovery within 90-110 % of serum value or slope 0.9-1.1. Freeze only once.
- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
- Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement.
- 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.
- Avoid grossly hemolytic, lipemic or turbid samples.
- Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
- 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.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2-8°C, under which conditions the wells will remain stable for 2 months, or until the labelled expiry date, whichever is earlier. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labelled expiry date, whichever is earlier.

Procedure:

Reagent preparation:

Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use. Adjust the incubator to 37 °C. Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature. Do not use Substrate if it looks blue. Do not use reagents that are contaminated or have bacteria growth.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators: Add 25ul of Calibrators and Samples to each well.

STEP 3

Addition of Enzyme Conjugate: Add 100ul of the Enzyme Conjugate solution to each well. Shake the plate for 30 seconds to ensure that the added components are well mixed.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer of 350ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of the Substrate: Add 100ul of Substrate Solution to each well. Mix gently for 5 seconds.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 50ul of the Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

STEP 9

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 30 minutes of adding the Stop Solution. Note down the absorbances. **The washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.**

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in mIU/ml in X axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.017	0.0 mIU/ml
CAL B	0.172	5.0 mIU/ml
CAL C	0.545	20.0 mIU/ml
CAL D	1.178	50.0 mIU/ml
CAL E	2.139	100.0 mIU/ml
CAL F	3.114	200.0 mIU/ml

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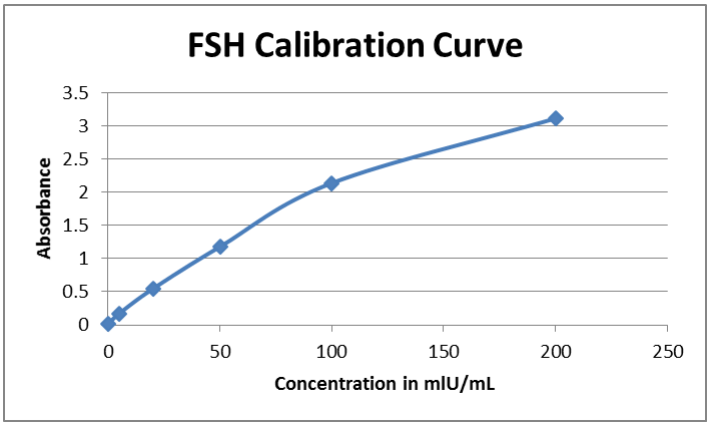

Limitations - interference

- The assay is unaffected by icterus (bilirubin < 1094 µmol/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).
- Criterion: Recovery within ± 10 % of initial value.
- 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.
- No interference was observed from rheumatoid factors up to a concentration of 2250 IU/mL.
- There is no high-dose hook effect at FSH concentrations up to 2000 mIU/mL.
- In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

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: 0.02 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 = 20).

Dilution

Not necessary due to the broad measuring range.

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
- Postmenopause: 22 - 146 mIU/mL

Studies with the FSH assay have revealed the following FSH values:

Test subjects	N	FSH (mIU/mL)		
		Percentile		
		50th	5th	95th
Men	404	4.8	1.5	13
Women				
- Follicular phase	399	7	4.1	11.1
- Ovulation phase	145	12.5	5.1	21.5
- Luteal phase	416	3.3	1.5	8
- Postmenopause	209	71.4	25	135

LH/FSH quotient: Quotients have been calculated from the results obtained with the LH assay and the 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.

Specific performance data

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

Precision

Precision was determined using 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 FSH assay (y) with the Roche Cobas FSH (x) using clinical samples gave the following correlations:

Number of samples measured: 158

Linear regression

y = 0.9874x + 0.083

r = 0.9855

The sample concentrations were between approx. 0 and 187 mIU/mL.

Analytical specificity

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

References

1. Johnson MR, Carter G, Grint C, et al. Relationship between ovarian steroids, gonadotropin and relaxin during the menstrual cycle. Acta Endocrinol 1983;129/2:121-125.
2. Beastall GH, Ferguson KM, O'Reilly DSJ, et al. Assays for follicle stimulating hormone and luteinizing hormone: Guidelines for the provision of a clinical biochemistry service. Ann Clin Biochem 1987;24:246-262.
3. Runnebaum B, Rabe T. Gynäkologische Endokrinologie und Fortpflanzungsmedizin Springer Verlag 1994. Band 1:17,253-255, Band 2:152-154,360,348. ISBN 3-540-57345-3, ISBN 3-540-57347-X.
4. Schmidt-Mathiesen H. Gynäkologie und Geburtshilfe. Schattauer Verlag 1992.
5. Scott MG, Ladenson JH, Green ED, et al. Hormonal evaluation of female infertility and reproductive disorders. Clin Chem 1989;35:620-630.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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