FSH (Follicle Stimulating Hormone) ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIAFSH1	FSH ELISA	96 Tests

Intended Use:

The FSH ELISA is intended to be used for the quantitative determination of FSH in human serum. This kit 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 oligospermic males usually have elevated 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 operates under the sandwich ELISA principle. The assay system uses one anti-FSH antibody immobilized on the solid phase and another mouse monoclonal anti-FSH antibody in the enzyme conjugate reagent. The test sample is allowed to react simultaneously with the antibodies resulting in FSH molecules present in the sample being sandwiched between the solid phase and enzyme linked antibodies. After incubation, the wells are washed to remove unbound sample material and conjugate. A substrate solution is added which results in a chromogenic reaction catalysed by the HRP conjugated to the detection antibody giving rise to development of a blue colour. The reaction is stopped with the addition of acidic reagent and the colour is changed to yellow. The colour intensity is proportional to the concentration of FSH in the sample and can be measured spectrophotometrically.

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 plastic sealable bag provided 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 (mIU/ml) made up in a human serum matrix. The exact concentrations are provided on the vial labels. 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 HRP labelled monoclonal Anti-FSH antibody in buffered saline. Once open, stable for 2 months at 2 - 8°C.
Wash Buffer Concentrate (40X)	1x25ml	PBS-Tween at pH 7.4. 40X concentrate. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x11ml	TMB and hydrogen peroxide reagent. Ready to use. Once open, stable for 2 months at 2 - 8°C.
Stop Solution	1x6ml	Diluted sulphuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2 -8°C.

IFU, resealable bag, plate covers.

Materials required but not provided:

Distilled water, micropipettes, incubator, 96-well plate reader and 96-well plate washer, absorbent paper.

Sample Collection:

Collect serum samples by separation from red blood cells after standard venepuncture technique. Store samples at 15 - 25°C for up to 8 hours, for 3 days at 2 - 8oC and 1 month at -20oC, under which conditions total T4 will be stable with a recovery within 90-110%. Avoid more than one freeze-thaw. Some sample collection tubes may contain differing substances which could affect the test result. If samples contain precipitate centrifuge before use. Do not use heat-inactivated samples. Do not use samples and controls stabilised with azide. Avoid grossly haemolytic, lipemic or turbid samples.

Storage and Stability:

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

Procedure:

Reagent preparation:

Ensure samples, calibrators, and controls are at room temperature (15 - 25 °C) before beginning the assay. Mix all reagents gently before use. Prepare wash solution concentrate by adding the contents of the bottle to 975 ml distilled water or dilute a portion by 1/40. Stable for 2 months at room temperature. Do not use Substrate if it looks blue. Do not use reagents that are contaminated.

STEP 1

Preparation: Remove the number of wells required and assign each well for the calibrators, controls and samples.

STEP 2

Addition of Samples: Add 25 µl of calibrators, controls and samples to each well.

STEP 3

Addition of Enzyme Conjugate: Add 100 µl of Enzyme Conjugate to each well. Tap the side of the plate gently to ensure that the added components are well mixed.

STEP 4

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

STEP 5

Washing: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. Use of an automated microplate strip washer is recommended. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 6

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

STEP 7

Incubation: Cover the plate with a plate cover and incubate for 20 minutes at room temperature (15 - 25°C). Ensure that this incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 50 µl of the Stop solution into each well Tap the side of the plate gently till the solution changes to completely from blue to yellow.

STEP 9

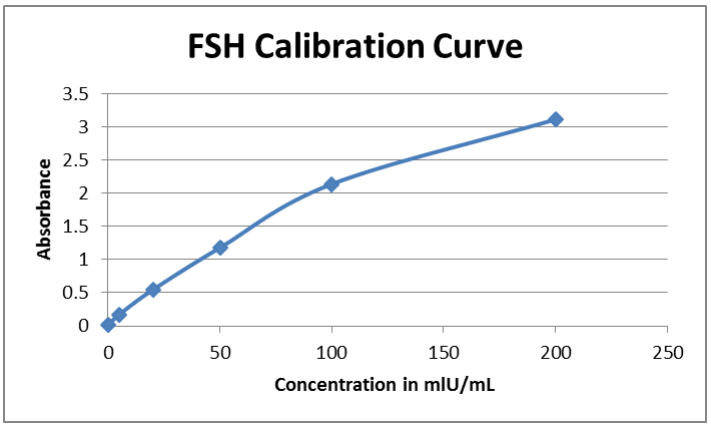
Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 30 minutes of adding the Stop Solution. Note down the absorbances.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance on the y axis and concentration in mIU/ml on the 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 Only:

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 mIU/ml
CAL D	1.178	50 mIU/ml
CAL E	2.139	100 mIU/ml
CAL F	3.114	200 mIU/ml



Limitations:

The washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.

Kit failure may result from using kits beyond the expiry date, poor washing procedures, contaminated reagents, improper operation of equipment, sample collection issues or timing errors.

This kit is intended only for testing of serum samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled blood.

Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop human anti-mouse antibodies (HAMA). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.

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The result from this test should not be used as the sole criteria for the diagnosis of patient fertility status or treatment, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

Performance:

Interference
The assay is unaffected by icterus (bilirubin < 1094 µmol/l or < 64 mg/dl), haemolysis (Haemoglobin < 0.621 mmol/l or < 1.0 g/dl), lipemia (Intralipid < 1900 mg/dl) and biotin (< 246 nmol/l or < 60 ng/ml) based on Recovery within ± 10 % of initial value. No interference was observed from rheumatoid factors up to a concentration of 2250 IU/ml
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.
There is no prozone 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.

Measuring range
0.2 - 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 Detection Limit
0.02 mIU/ml
The detection limit represents the lowest analyte level that can be distinguished from zero.

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.

Precision
Precision was determined using reagents, pooled human sera, and controls testing 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 another commercially available FSH ELISA (x) using clinical samples (n = 158) gave the following correlation:
y = 0.987 x + 0.083, r = 0.986
The sample concentrations were approximately between 0 - 187 mIU/ml.

Analytical specificity
For the monoclonal antibodies used, the following cross-reactivities were found:
LH 0.045, hGH and hCG no cross-reactivity.

References

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

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