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 by professionals 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 seminiferous 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 works via a sandwich principle ELISA. The assay system comprises one anti-FSH antibody on the solid phase and mouse monoclonal anti-FSH antibody in the Enzyme Conjugate solution. Calibrators, Controls and samples are added to assigned wells along with the conjugate. The samples interact with both the solid phase and the conjugate antibodies resulting in any FSH present being bound to and immobilized on the solid phase and labelled by the enzyme linked antibodies during the incubation step. After, the wells are washed thoroughly to remove unbound conjugate. Substrate solution is added which uses the HRP enzyme on the labelled immunocomplexes to catalyse the oxidation of the substrate resulting in the development of a blue coloured product. The reaction is stopped on addition of acid Stop Solution which also changes the blue colour to yellow, the intensity of which can be measured spectrophotometrically and is directly proportional to the concentration of FSH in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with polyclonal anti FSH antibody. 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 till expiry at 2 - 8°C if stored as described above.
FSH Calibrators	6x1ml	6 vials containing FSH in a human serum matrix. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABELS. Referenced against the WHO 2nd IRP, HMG). Once open stable for till expiry at 2 - 8°C.
Enzyme Conjugate	1x12ml	1 vial containing HRP labelled monoclonal Anti-FSH antibody in buffered saline. Once open, stable till expiry at 2 - 8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. Once diluted it is stable at room temperature for one month.
Substrate Solution	1x12ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable till expiry at 2 - 8°C.
Stop Solution	1x12ml	Hydrochloric Acid solution (2N) Ready to use. Once open, stable till expiry at 2 - 8°C.

IFU, Plate covers.

Materials required but not provided:

Distilled water, pipettes, disposable tips, incubator, absorbent paper, microplate reader and microplate washer.

Specimen Collection:

Collect serum by separation after standard venepuncture technique. FSH is stable in serum up to 7 days at 2 - 8 °C, up to 1 month at -20 °C and up to 8 hours at 18 - 25 °C. Fibrin may interfere in the assay so ensure that full clot formation has taken place before centrifugation to remove red blood cells. Remove any sediment or precipitated material in serum samples by centrifugation before use. Do not use grossly haemolytic, lipemic or icteric samples.

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 with desiccant provided and return to 2 - 8°C, under which conditions the wells will remain stable until the labelled expiry date. Seal and return all the other unused reagents to 2 - 8°C, under which conditions they will remain stable until the expiry date.

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. Prepare wash solution concentrate by adding the contents of the Wash Buffer Concentrate bottle to 735 ml of distilled water. Stable for 2 months at room temperature. Do not use reagents that are contaminated or have bacterial growth.
If calibrators are supplied lyophilised, reconstitute each vial with 0.5 ml distilled water. Leave to stand for 20 minutes then mix well before use. Store at 2 - 8°C.

STEP 1

Preparation: Remove the number of wells required and assign calibrators, controls and samples to well positions for the assay run.

STEP 2

Addition of samples and calibrators: Add 50 µl of calibrators, controls and samples to assigned wells.

STEP 3

Addition of Enzyme Conjugate: Add 100 µl of 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 room temperature (15 - 25°C).

STEP 5

Wash Step: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with 350 µl of diluted wash buffer leaving the plate to soak for one minute for each wash. After the final washing cycle, invert the plate onto blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of the Substrate: Add 100 µl 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 carried out in the dark.

STEP 8

Stopping the Reaction: Add 100 µl of Stop Solution to each well. Shake the plate gently to mix until the well contents change 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. Record the absorbance for each well.

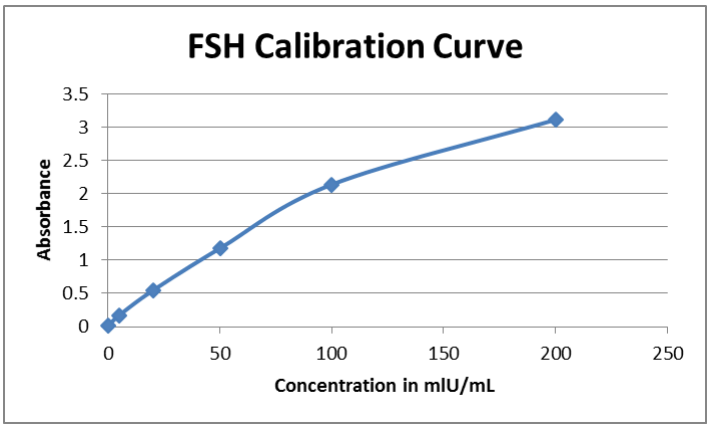
The washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the Absorbance on the y axis and FSH Concentration in mIU/ml on the x axis.
- Draw a point to point curve through the plotted points on a linear graph paper. Determine the concentration of Quality Controls and unknown samples from the graph using the mean Absorbance value for each.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.007	0.0 mIU/ml
CAL B	0.095	5.0 mIU/ml
CAL C	0.286	20.0 mIU/ml
CAL D	0.669	50.0 mIU/ml
CAL E	1.307	100.0 mIU/ml
CAL F	2.584	200.0 mIU/ml



Prestige Diagnostics UK Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100
www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

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Limitations:

The result from this test should not be used as the sole criteria for diagnosis, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
Enzyme immunoassays potentially demonstrate interference by samples containing rheumatoid factor and antinuclear antibodies, and samples from patients receiving treatments containing mouse monoclonal antibodies. The reagents have been formulated to reduce such interference, but the effects may not be eliminated completely.

Expected values

Men: 0 - 20 mIU/ml
Women:

Follicular phase: 0 - 20 mIU/ml
Ovulation phase: 15 - 30 mIU/ml
Luteal phase: 0 - 20 mIU/ml
Post menopause: 40 - 200 mIU/ml

Performance Characteristics:

Precision
Intra-Assay Precision (n = 20):

Sample	Mean	SD	CV %
Pool 1	9.17	0.54	5.93
Pool 2	21.04	1.24	5.90
Pool 3	50.62	2.16	4.27

Inter-Assay Precision (n = 20):

Sample	Mean	SD	CV %
Pool 1	8.71	0.65	7.50
Pool 2	20.09	1.82	9.04
Pool 3	55.42	4.29	7.75

Measuring Range
2.5 - 200 mIU/ml.

Analytical Sensitivity
Lowest detection limit: 2.0 mIU/ml

Dilution
Should not be required due to the broad measuring range.

Method comparison
A comparison of the FSH ELISA (y) with a predicate FSH assay (x) using clinical samples (n = 124) gave the following correlation:

$y = 1.01\,x + 0.340,\ r = 0.980$

Prozone
Prozone effect was not seen in the FSH ELISA assay up to a concentration of 3000 mIU/ml FSH.

Cross Reactivity
Some potentially interfering antigens were spiked into samples at the concentrations stated and tested in the FSH ELISA, the following cross-reactivities were found:

Interferent	Concentration	FSH Result	% Cross Reactivity
hCG	500000 mIU/ml	0.0 mIU/ml	0.0
LH	500 mIU/ml	0.0 mIU/ml	0.0
TSH	500 IU/ml	0.0 mIU/ml	0.0
Prolactin	500 mg/ml	0.5 mIU/ml	0.1

References:

1. Marshall JC. Clin in Endocrinol Metab 1975; 4: 545.
2. Cohen KL. Metabolism 1977; 26: 1165.
3. Rebar RW, Erickson GF and Yen SSC. Fertil Steril 1982; 37: 35.
4. Abraham GE. Ed. Radioassay Systems in Clinical Endocrinology. Marcel Dekker, Inc. New York 1981.
5. Uotila M, Ruoslahti E and Engvall E. J Immunol Methods 1981; 42: 11.

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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Prestige Diagnostics UK Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100
www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

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