

PATHOZYME® FOLLICLE STIMULATING HORMONE ^{Ref} OD337

Enzyme-Immunoassay (EIA) for the quantitative determination of FSH

in human serum

Store at 2°C to 8°C. DO NOT FREEZE.

For in-vitro use only.

INTRODUCTION

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 synthesise and secrete male and female sex hormones. The levels of circulating FSH and LH are controlled by the sex hormones via a negative feedback loop.

FSH is a glycoprotein secreted by 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, Thyroid Stimulating Hormone (TSH) and Human Chorionic Gonadotropin (hCG), FSH consists of alpha and beta subunits. As the alpha subunits are similar structurally, the biological and immunological properties of each are dependent on the unique beta subunit.

In females, 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 these cells and stimulates steroidogenesis. Increased intraovarian oestradiol production occurs as follicular maturation advances, in turn stimulating increased FSH receptor activity and FSH follicular binding. FSH, LH and oestradiol are therefore related in supporting ovarian maturation in women.

FSH levels are elevated after menopause, castration, and premature ovarian failure. The levels of FSH may be normalised through the administration of oestrogen, which demonstrates a negative feedback mechanism. Abnormal relationships between FSH and LH, and FSH and oestrogen have been linked to anorexia nervosa and polycystic ovarian disease.

Although there are exceptions, ovarian failure is normally indicated when random FSH concentrations exceed 40mIU/ml.

FSH regulates the growth of seminiferous tubules and maintains spermatogenesis in men. However, androgens, unlike oestrogen, 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 levels of FSH. Tumours of the testes usually depress serum 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 following preparations were tested as negative: HCG (WHO 1st International Reference Preparation (75/539) at 100,000 mIU/ml, TSH (WHO 2nd International Reference Preparation (80/558) at 100mIU/ml, LH (WHO 1st International Reference Preparation 68/40) at 500 mIU/ml, Prolactin (WHO 1st International Reference Preparation (75/504) at 500ng/ml and HGH (WHO 1st International Reference Preparation 65/217) at 200ng/ml.

INTENDED USE

PATHOZYME FSH is an Enzyme Immunoassay (EIA) for the quantitative determination of Follicle Stimulating Hormone (FSH) in human serum. For professional use only.

PRINCIPLE OF THE TEST

Monoclonal, anti-FSH antibodies are prepared, purified and coated onto microtitre wells. Test sera are applied. Then another monoclonal anti-FSH labelled with Horseradish Peroxidase enzyme is added. If human FSH is present in the sample, it will combine with the antibody on the well and the enzyme Conjugate, resulting FSH molecules being "sandwiched" between the solid phase and the enzyme linked antibodies. After incubation, the wells are washed with distilled water to remove unbound labelled antibodies.

On addition of the Substrate (TMB), a colour will develop only in those wells in which enzyme is present, indicating the presence of FSH. The reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450nm. The concentration of FSH is directly proportional to the colour intensity of the test sample.

This test has been calibrated against WHO, 2nd IRP, 78/549.

^{Ref}
OD337



12 x 8 wells x 1

CONTENTS

Microtitre Plate

Breakable wells coated with specific antibody contained in a resealable foil bag with a desiccant.

Cal	A	0 mIU/ml
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Reference Standard: Human serum free of FSH. Lyophilised.

1

Cal	B	5 mIU/ml
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1

Reference Standard: FSH diluted in human serum. Lyophilised.

Cal	C	15 mIU/ml
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1

Reference Standard: FSH diluted in human serum. Lyophilised.

Cal	D	50 mIU/ml
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1

Reference Standard: FSH diluted in human serum. Lyophilised.

Cal	E	100 mIU/ml
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1

Reference Standard: FSH diluted in human serum. Lyophilised.

Cal	F	200 mIU/ml
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1

Reference Standard: FSH diluted in human serum. Lyophilised.

Conj

11 ml

Anti-FSH HRP Conjugate: Anti-FSH conjugated to HRP. Ready to use (Pink)

Subs	TMB
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11 ml

Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)

Soln	Stop	HCl	1M
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11 ml

Stop Solution: Hydrochloric Acid diluted in purified water. Ready to use. (Colourless)

Instruction Leaflet and EIA Data Recording Sheet

1 + 1

MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes: 100µl, 200µl and 1000µl

Disposable pipette tips

Absorbent paper

Microplate reader fitted with a 450nm filter

Graph paper

Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME FSH contains materials of human origin which have been tested and confirmed negative for HCV, HIV I and II antibodies and HBsAg by FDA approved methods at single donor level. Because no test can offer complete assurance that products derived from human source will not transmit infectious agents it is recommended that the reagents within this kit be handled with due care and attention during use and disposal. All reagents should, however, be treated as potential Biohazards in use and for disposal. Do not ingest.

PATHOZYME FSH Reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

PATHOZYME FSH Stop Solution is dilute Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

PATHOZYME FSH reagents contain 1.0% Proclin™ 300* as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with running water and seek medical advice.

* Proclin™ 300 is a Trade Mark of ROHM and HAAS Limited.

STORAGE

Reagents must be stored at temperatures between 2°C to 8°C.

Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS (except Standards for storage) as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversely affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at -20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

Add 1ml of distilled water to each standard vial in order to reconstitute the lyophilised standards. Allow to stand for at least 20 minutes then mix gently. Reconstituted standards will be stable for up to 30 days when stored at 2-8°C. For longer storage store sealed at -20°C when not in use. Thawed standards must be mixed prior to testing.

LIMITATIONS OF USE

The use of samples other than serum has not been validated in this test. There is no reuse protocol for this product. When making an interpretation of the test it is strongly advised to take all clinical data into consideration. Diagnosis should not be made solely on the findings of one clinical assay.

ASSAY PROCEDURE

1. Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assay.
2. One set of Standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.
4. Dispense 50µl of Standards and test serum into the assigned wells.
5. Dispense 100µl of Anti-FSH Conjugate into each well.
4. Thoroughly Mix for 30 seconds. It is very important to mix completely.
5. Incubate the plate for 45 minutes at room temperature (20°C to 25°C).
6. At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the Biohazard container.
7. Hand Washing: Fill the wells with a minimum of 300µl of distilled water per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper.
8. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
9. Machine Washing: Ensure that 300µl of distilled water is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 5 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
10. Dispense 100µl Substrate Solution into each well and mix gently for 5 seconds.
11. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
12. Stop the reaction by adding 100µl Stop Solution to each well.
13. Gently mix for 30 seconds to ensure that the blue colour changes completely to yellow colour.
14. Read the optical density immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

TROUBLESHOOTING

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

Use a separate disposable tip for each sample to prevent cross contamination.

Duplication of all standards and specimens, although not required, is recommended.

Specimens and standards should be run at the same time to keep testing conditions the same.

It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used, since pipetting of all Standards and specimens

should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available.

Replace caps on all reagents immediately after use.

Avoid repeated pipetting from stock reagents as this is likely to cause contamination.

Do not mix reagents or antibody coated strips from different kits. When dispensing, care should be taken not to touch the surface of the well.

Do not allow reagent to run down the sides of the well. Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.

Once an assay has been initiated, the wells should not be allowed to become dry during the assay.

Do not contaminate the Substrate Solution as this will render the whole kit inoperative.

Check the precision and accuracy of the laboratory equipment used during the procedure to ensure reproducible results.

The unused strips should be resealed in the foil bag, containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

CALCULATION OF RESULTS

Calculate the mean absorbance value (A^{450}) for each set of Standards and test serum. Construct a standard curve by plotting the mean absorbance from each Standard against its concentration in mIU/ml on graph paper, with absorbance values on the Y-axis and concentrations on the X-axis.

Use the mean absorbance values for each specimen to determine the corresponding concentration of FSH in mIU/ml from the standard curve.

If levels of controls or users known samples do not give expected results, test results must be considered invalid.

If using a software package choose a quadratic regression curve fit.

EXPECTED VALUES AND SENSITIVITY

The graph produced by the Calibrators should be Hyperbolic in shape with the OD450 of the Calibrators proportional to their concentration. The OD of Calibrator A should be less than 0.75 and the OD of Calibrator F should be greater than 1.5 for the assay results to be valid. Based on random selected out-patient clinical laboratory samples, the mean FSH values in males (N=100) and females (N=150) are 11 and 12 mIU/ml respectively.

The mean FSH values in post-menopausal (N=60) and pregnant women (N=60) are 94 and 1.0mIU/ml respectively. The minimum detectable concentration of **PATHOZYME FSH** is estimated to be 1.5mIU/ml.

No prozone (Hook) effect is observed in this assay at levels up to 1,500 mIU/ml.

EVALUATION DATA

Calibrated to major competitors and in house standards.

The co-efficient of variation of **PATHOZYME FSH** is less than or equal to 10%.

In an evaluation between the Omega Pathozyme FSH kit and the Serono Serozyme FSH kit for samples with levels between 0.1 and 143 mIU/ml the following data was generated.

Number of Samples	136
Correlation Co-efficient	0.99
Slope	1.05
Intercept	0.55
Omega Mean	18.8 mIU/ml
Serono Mean	18.4 mIU/ml

These kits were shown to give good correlation.

REFERENCES

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- (2) **Cohen, K. L.** *Metabolism.* 1977;26:1165.
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- (4) **Abramham, G. E.** (ed.). *Radioassay Systems in the Clinic. Endocrinol.* Marcel Dekker Inc., New York: 1981.
- (5) **Uotila, M., Ruoslahti, E. and Engvall, E. J.** *Immunol. Methods.* 1981;42:11.

QUICK REFERENCE TEST PROCEDURE

1. Dispense 50µl of Standards or samples and 100µl of Anti-FSH Conjugate into each well and mix thoroughly for 30 seconds.
2. Incubate for 45 minutes at room temperature (20°C to 25°C).
3. Discard well contents and wash five times with distilled water.
4. Add 100µl Substrate Solution into each well and gently shake for 5 seconds.
5. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
6. Add 100µl of Stop Solution to each well and gently shake for 30 seconds.
7. Read the Optical Densities immediately (no later than 10 minutes) using microplate reader with a 450nm filter.

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