

PATHOZYME[®] THYROID STIMULATING HORMONE Ref OD387

Enzyme Immunoassay for the quantitative determination of TSH

in human serum

Store at 2°C-8°C. DO NOT FREEZE.

For in-vitro diagnostic use only.

INTRODUCTION

The determination of serum or plasma levels of Thyroid Stimulating Hormone (TSH) is recognised as a sensitive method in the diagnosis of Primary and Secondary Hypothyroidism. TSH is secreted from the anterior lobe of the Pituitary gland and induces the production of Thyroxine (T4) and Triiodothyronine (T3) from the Thyroid gland. Structurally, TSH is a 28,000 dalton glycoprotein consisting of chemically different alpha and beta chains.

Although the normal level of TSH in the blood is extremely low, it is essential for the normal regulation of the Thyroid gland. TSH release is regulated by a TSH-Releasing Hormone (TRH) produced by the Hypothalamus. The levels of TSH and TRH are inversely related to the level of Thyroid Hormone. When there are high levels of TSH in the blood, less TRH is released by the Hypothalamus and in turn less TSH is secreted by the Pituitary. This process is known as a negative feedback mechanism and is responsible for the maintenance of proper TSH levels in the blood.

TSH and the Pituitary glycoproteins: Luteinising Hormone (LH), Follicle Stimulating Hormone (FSH) and human Chorionic Gonadotropin (hCG) all have identical alpha chains. In each case, the beta chain is distinct, although there are identical amino acid sequences which can cause considerable cross reactivity with some polyclonal TSH antisera.

The use of monoclonal antibodies in **PATHOZYME TSH** eliminates this interference, which could result in falsely elevated TSH values in either menopausal or pregnant females; a population whose evaluation of thyroid status is clinically significant.

The following preparations were tested as negative: HCG (WHO 2nd International Standard 61/2) at 200,000 mIU/ml, FSH (WHO 2nd International Reference Preparation HMG) at 200 mIU/ml, LH (WHO 1st International Reference Preparation 75/504) at 200 ng/ml and HGH (WHO 1st International Reference Preparation 65/217) at 200ng/ml.

INTENDED USE

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

PRINCIPLE OF THE TEST

Specific anti-TSH antibodies are coated onto microtitration wells. Test sera are applied. Then goat anti-TSH labelled with Horseradish Peroxidase enzyme (Conjugate) is added. If human TSH is present in the sample it will combine with the antibody on the well and the enzyme Conjugate, resulting in the TSH molecule being sandwiched between the solid phase and the enzyme linked antibodies. After incubation, the wells are washed to remove unbound labelled antibodies. On addition of the Substrate (TMB), a colour will develop only in those wells in which the enzyme Conjugate is present, indicating the presence of TSH. The enzyme reaction is stopped by the addition of dilute Hydrochloric acid and the absorbance is then measured at 450nm.
This test has been calibrated to the NIBSC Thyroid Stimulating Hormone 2nd International Reference Preparation 1983 80/558.

CONTENTS

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Microtitre Plate	12 x 8 wells x 1
Breakable wells coated with specific antibodies contained in a resealable foil bag with a desiccant.	
Cal A 0 μ IU / ml	1 ml
Reference Standard: Human serum free of TSH. Lyophilised. (Colourless)	
Cal B 0.5 μ IU / ml	1ml
Reference Standard: TSH diluted in human serum. Lyophilised. (Colourless)	
Cal C 2 μ IU / ml	1ml
Reference Standard: TSH diluted in human serum. Lyophilised. (Colourless)	
Cal D 5 μ IU / ml	1ml
Reference Standard: TSH diluted in human serum. Lyophilised. (Colourless)	
Cal E 10 μ IU / ml	1ml
Reference Standard: TSH diluted in human serum. Lyophilised. (Colourless)	
Cal F 25 μ IU / ml	1ml
Reference Standard: T4 diluted in human serum. Lyophilised. (Colourless)	
Washbuf 20X	50ml
Wash Buffer concentrate: Tris based buffer containing detergents. (Colourless)	
Conj	11 ml
TSH HRP Conjugate: TSH conjugated to Horseradish Peroxidase. Ready to use. (Red)	
Subs TMB	11 ml
Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)	
Soln Stop HCl 1M	11ml
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: 100 μ l, 200 μ l, 1000 μ l and 5000 μ l
Disposable pipette tips
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

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

PATHOZYME TSH reagents contain 1% ProclinTM 300* as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with running water and seek medical advice.

ProclinTM 300 is a trade mark of ROHM & 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.

Standards: Add 1 ml of distilled water to each Standard vial in order to reconstitute the lyophilised standards. Allow to stand for a minimum of 20 minutes before use. Rehydrated standards will be stable for up to 30 days when stored at 2°C to 8°C. For longer storage store sealed at -20°C when not in use. Thawed Standards must be mixed gently prior to use.

Wash Buffer:
Dilute the concentrated Wash Buffer using 1 part Wash Buffer concentrate with 19 parts distilled water. For every 8-well breakable strip, prepare 25ml of diluted Wash Buffer by adding 1.25ml of concentrated Wash Buffer to 23.75ml of distilled water. Prepare fresh diluted Wash Buffer prior to every assay run. Extra Wash Buffer is supplied to enable priming of automatic washing machines.

The washing procedure is critical to the outcome of this test. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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 100µl of Standards and test serum into the appropriate wells.
5. Dispense 100µl of Anti-TSH Conjugate into each well. Mix thoroughly for 30 seconds. It is very important to mix completely.
6. Incubate for 60 minutes at room temperature (20°C to 25°C).
7. Hand Washing: 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.
8. Fill the wells with a minimum of 300µl of wash buffer per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Wash the empty wells 5 times.
9. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
10. Machine Washing: Ensure that 300µl of wash buffer 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.
11. Dispense 100µl Substrate Solution into each well and mix gently for 5 seconds.
12. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
13. Stop the reaction by adding 100µl Stop Solution to each well.
14. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
15. 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 specimens. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration in µU/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 TSH in µU/ml from the standard curve.

If levels of Calibrators 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 0.2 and the OD of Calibrator F should be greater than 1.5 for the assay results to be valid. Normal values for adults between the ages of 21 and 54 years is 0.4 to 4.2µU/ml rising to 0.5 to 8.9 µU/ml between the ages of 55 and 87. During Pregnancy the normal ranges are as follows: 1st Trimester 0.3 to 4.5µU/ml, 2nd Trimester 0.5 to 4.6µU/ml and 3rd Trimester 0.8 to 5.2µU/ml. Concentrations of 1,000µU/ml have been observed using **PATHOZYME TSH** with no prozone (Hook) effect.

EVALUATION DATA

Calibrated to major competitors and in house standards.

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

In an evaluation between the Omega Pathozyme TSH kit and the DPC Immulite 2000 TSH Kit for samples with levels between 0.4 and 63 µU/ml the following data was generated.

Number of Samples	59
Correlation Co-efficient	0.994
Slope	1.027
Intercept	0.787
Omega Mean	8.39 µU/ml
DPC Mean	9.41 µU/ml

These kits were shown to give good correlation.

REFERENCES

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3. Uotila, M., Rouslahti, E., and Engvall, E. *J. Immunol. Methods* 1981;42:11-15.
4. Burger, H.G., and Patel, Y.C. Thyrotropin releasing hormone-TSH. *Clinic. Endocrinol. Metab.* 1977;6:831.
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QUICK REFERENCE TEST PROCEDURE

1. Dispense 100µl of test serum or Standards and 100µl Enzyme Conjugate into each well. Gently mix for 30 seconds.
2. Incubate for 60 minutes at room temperature (20°C to 25°C).
3. Discard well contents and wash 5 times with wash buffer.
4. Add 100µl of Substrate Solution to each well. Gently shake for 5 seconds.
5. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
6. Add 100µl Stop Solution to each well and gently shake for 30 seconds.
7. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

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