

PATHOZYME[®] TOTAL THYROXINE (T4) Ref OD377

Enzyme-Immunoassay (EIA) for the detection of Thyroxine (T4) in human serum

Store at 2°C to 8°C. DO NOT FREEZE
For in-vitro use only

INTRODUCTION

Thyroxine (3, 5, 3,5 -tetraiodothyronine) or T4 is the most commonly measured thyroid hormone for diagnosis of thyroid function. It is synthesised in the follicles of the thyroid gland and has its primary influence on oxygen consumption and heat production in virtually all tissues. It is also important for growth development and for sexual maturation of growing mammals. Greater than 99.9% of T4 transported through the bloodstream is bound to plasma proteins. The major binding protein being Thyroxine Binding Globulin (TBG) and the secondary binding proteins being Thyroxine Binding Albumin and Prealbumin. Primary Hypothyroidism results in decreased production of T4 by the thyroid gland and consequently an abnormally low circulating T4 concentration in the blood. Primary Hyperthyroidism leads to excessive thyroid production of T4 and results in elevated T4 concentrations in the blood.

PATHOZYME T4 assay provides a rapid and sensitive method for measuring T4 in human serum using highly specific T4 monoclonal antibody and a T4 enzyme labelled conjugate solution.

INTENDED USE

PATHOZYME T4 is an Enzyme Immunoassay (EIA) for the quantitative determination of Thyroxine in human serum. For professional use only.

PRINCIPLE OF THE TEST

Specific sheep anti-T4 antibodies are coated onto microtitration wells. Test sera are applied. T4 with Horseradish Peroxide enzyme (Conjugate) is added which competes with the released serum T4 for available binding sites on the solid phase.

After incubation, the wells are washed with water to remove any unbound T4 or T4 enzyme conjugate. On addition of the Substrate (TMB), a colour develops only in those wells in which enzyme is present, indicating a lack of serum T4. The reaction is stopped by the addition of dilute Hydrochloric acid and the absorbance is then measured at 450nm.

This test has been calibrated against in house standards. There is no international standard for this test.

CONTENTS

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OD377



12 x 8 wells x 1

Microtitre Plate			
Breakable wells coated with specific antibodies contained in a resealable foil bag with a desiccant.			
Cal A	0 ng/ml	1 ml	
Reference Standard: Human serum free of T4. Ready to use. (Colourless)			
Cal B	20 ng/ml	1ml	
Reference Standard: T4 diluted in human serum. Ready to use. (Colourless)			
Cal C	50 ng/ml	1ml	
Reference Standard: T4 diluted in human serum. Ready to use. (Colourless)			
Cal D	100 ng/ml	1ml	
Reference Standard: T4 diluted in human serum. Ready to use. (Colourless)			
Cal E	150ng/ml	1ml	
Reference Standard: T4 diluted in human serum. Ready to use. (Colourless)			
Cal F	250 ng/ml	1ml	
Reference Standard: T4 diluted in human serum. Ready to use. (Colourless)			
Washbuf	20X	50ml	
Wash Buffer concentrate: Tris based buffer containing detergents. (Colourless)			
Conj	11X	1.3ml	
T4 HRP Conjugate concentrate: T4 conjugated to Horseradish Peroxidase. (Colourless)			
DIL	Conj	12ml	
Conjugate Diluent: Phosphate based buffer containing stabilising proteins. Working Strength (Green)			
Subs	TMB	11ml	
Substrate Solution: 3,3q 5,5qTetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)			
Soln	Stop	HCl	1M
Stop Solution: Hydrochloric acid diluted in purified water. Ready to use. (Colourless)			
Instruction leaflet and EIA Data Recording Sheet			

MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes: 50µl, 100µl, 200µl and 1000µl
Disposable pipette tips
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME T4 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 T4 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 T4 Stop Solution is dilute Hydrochloric acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

PATHOZYME T4 reagents contain 1% 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 & 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 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.

Conjugate: Dilute the concentrated conjugate using 1 part concentrated conjugate with 10 parts conjugated diluent. eg. add 0.1 ml concentrated conjugate to 1.0 ml of conjugate diluent. This should be done 20 minutes prior to initiation of the assay. Ensure that the diluted conjugate is at room temperature. Do not induce foaming. Use within 24 hours.

Only prepare sufficient working strength conjugate solution to perform the assays required for that day. ie. 2 strips of 8 wells will require 160 µl of concentrated conjugate diluted in 1.6 ml of conjugate diluent.

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.

T4 levels have been reported to be influenced by the following conditions and treatments: High TSH levels, pregnancy, oestrogen therapy, oral contraceptives, heparin, phenytoin propanolol.

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 25µl of standards and test serum into the assigned wells.
5. Dispense 100µl of working strength Conjugate into each well
6. Thoroughly mix for 30 seconds. It is very important to mix completely in this step.
7. Incubate for 60 minutes at room temperature (20°C to 25°C).
8. 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.
9. Hand Washing: 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.
10. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
11. 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.
12. Dispense 100µl Substrate Solution into each well and mix gently for 5 seconds.
13. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
14. Stop the reaction by adding 100µl Stop Solution to each well.
15. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
16. 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 (A450) for each set of standards and specimens. Construct a standard curve by plotting the mean absorbance from each standard against its concentration in ng/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 T4 in ng/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 polygon with data extrapolation curve fit.

EXPECTED VALUES AND SENSITIVITY

The graph produced by the calibrators should be Hyperbolic in shape with the OD450 of the calibrators inversely proportional to their concentration. The OD of Calibrator A should be greater than 1.5 and the OD of Calibrator F should be less than 0.75 for the assay results to be valid. **PATHOZYME T4** was performed in a study of 200 euthyroid patients in one geographical location and yielded a normal range of 50 to 130 ng/ml. This range corresponds to those suggested by other commercial manufacturers. It is recommended that laboratories adjust values to reflect geographical and population differences specific to patients they serve. The minimum detectable concentration of Thyroxine by **PATHOZYME T4** is estimated to be 5 ng/ml.

EVALUATION DATA

Calibrated to major competitors and in house standards.

The co-efficient of variation of **PATHOZYME T4** is less than or equal to 10%.

In an evaluation between the Omega Pathozyme Total T4 kit and the Abbott AxSym Total T4 Kit for samples with levels between 13 and 245 ng/ml the following data was generated.

Number of Samples	82
Correlation Co-efficient	0.9095
Slope	0.9136
Intercept	1.0488
Omega Mean	99 ng/ml
Abbott Mean	101 ng/ml

These kits were shown to give good correlation.

REFERENCES

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- (4) Larsen, P.R., Ingbar, S.H., William's Text Book of Endocrinology, Wilson JD and Foster eds., Philadelphia, Sanders Company, 1992, Section 3, Thyroid, Chapter 8, The Thyroid Gland, P. 358-487
- (5) Robbins, J. Radioassay and Thyroid Gland. Metabolism. 22:1021;1973.

QUICK REFERENCE TEST PROCEDURE

1. Dispense 25µl of test serum or Standards and 100µl of working strength conjugate into each well and mix thoroughly for 30 seconds.
2. Incubate for 60 minutes at room temperature (20°C to 25°C).
3. Discard well contents and wash five 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 of 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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