PATHOZYME® FREE TRIIODOTHYRONINE Ref OD457

Enzyme Immunoassay for the quantitative determination of fT3 in human serum.

Store at 2°C to 8°C. DO NOT FREEZE.

For in-vitro use only.

INTRODUCTION

L-Triiodothyronine a thyroid hormone, circulates in blood almost completely bound (>99.5%) to carrier proteins. The main transport protein is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of triiodothyronine is believed to be responsible for the biological action. Furthermore, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In individuals with normal thyroid function, as the concentrations of the carrier proteins change, the total T3 levels also change so that the free thiiodothyronine (fT3) concentration remains constant. Thus, measurements of fT3 concentrations correlate more reliably with clinical status than total triiodothyronine levels.
For example, the increase in total triiodothyronine levels associated with pregnancy, oral contraceptives, and oestrogen therapy result in higher total T3 levels while the fT3 concentration remains basically unchanged.

PATHOZYME FREE T3 Enzyme Immunoassay provides a rapid, sensitive method for measuring T3 in unextracted serum using T3 antibody and enzyme labelled conjugate.

INTENDED USE

PATHOZYME FREE T3 is an Enzyme Immunoassay (EIA) for the quantitative determination of Free Triiodothyronine (fT3) in human serum.
For professional use only.

PRINCIPLE OF THE TEST

The fT3 test is a solid phase competitive enzyme immunoassay. Patient serum samples, standards, and T3-Enzyme Conjugate are added to wells coated with monoclonal T3 antibody. fT3 in the specimen and the T3 labelled conjugate compete for available binding sites on the antibody. After incubation at room temperature, the wells are washed with distilled water to remove unbound T3 conjugate. On addition of the Substrate (TMB), a colour develops only in those wells in which enzyme is present, indicating a lack of serum fT3. The reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance measured at 450 nm.
This test has been calibrated against in house standards. There is no International standard for this test.

CONTENTS

Ref OD457

96

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 pg/ml	**1 ml**
Reference Standard: Human serum free of Free T3. Ready to use. (Colourless)	
Cal B Level as stated on vial	**1ml**
Reference Standard: Free T3 diluted in human serum. Ready to use. (Colourless)	
Cal C Level as stated on vial	**1ml**
Reference Standard: Free T3 diluted in human serum. Ready to use (Colourless)	
Cal D Level as stated on vial	**1ml**
Reference Standard: Free T3 diluted in human serum. Ready to use (Colourless)	
Cal E Level as stated on vial	**1ml**
Reference Standard: Free T3 diluted in human serum. Ready to use (Colourless)	
Cal F Level as stated on vial	**1ml**
Reference Standard: Free T3 diluted in human serum. Ready to use (Colourless)	
Conj	**10.5ml**
T3 HRP Conjugate: T3 conjugated to Horseradish Peroxidase. Ready to use. (Pink)	
Subs TMB	**11ml**
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: 100µl, 200µl, 1000µl and 5000µl
Disposable pipette tips
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME FREE T3 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 FREE T3 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 FREE T3 Stop Solution is dilute Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

PATHOZYME FREE T3 reagents contain 1.0% Proclin™ 300* as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with water and seek medical advice.

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

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 adversly 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.

LIMITATIONS OF PROCEDURE

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 assy.
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 standard, control or test serum into the assigned well.
5. Dispense 100µl of Triiodothyronine Conjugate Solution, to all wells. Swirl the microplate gently for 20 to 30 seconds to mix and cover.
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 distilled water 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 distilled water is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 5 times.
11. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
12. Dispense100µl of Substrate Solution to all wells.
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. It is important to make sure that all the blue colour changes completely to a yellow colour.
16. Read absorbance at 450 nm with a microtitre well reader within 10 minutes.

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 Reference Standards and specimens. Construct a standard curve by plotting the mean absorbance obtained from each Reference Standard against its concentration in pg/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 fT3 in pg/ml from the standard curve.
If levels of Calibrator 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

Based on random selected out-patient clinical laboratory samples, the normal range of fT3 is 1.4 – 4.2 pg/ml. The minimum detectable concentration of fT3 by **PATHOZYME FREE T3** is estimated to be 0.05 pg/ml.

Substance	Cross Reactivity (%) at 10µg/ml Triiodothyronine equivalent
Thiroxine	< 0.0002
Iodothyrosine	< 0.0001
Diiodothyrosine	< 0.0001
Diiodothyronine	< 0.0001
Phenylbutazone	< 0.0001
Sodium Salicylate	< 0.0001

EVALUATION DATA

The co-efficient of variation of **PATHOZYME fT3** is less than or equal to 10%.
In an evaluation between the Omega Pathozyme Free T3 kit and a coated tube radioimmunoassay analogue method for samples with levels between 0.1 and 14 pg/ml the following data was generated.

Number of Samples	85
Correlation Co-efficient	0.955
Slope	0.925
Intercept	0.15
Omega Mean	3.4 pg/ml
Radioimmunoassay Mean	3.5 pg/ml

These kits were shown to give good correlation
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.

Calibrated to major competitors and in house standards.

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QUICK REFERENCE TEST PROCEDURE

1. Dispense 50µl of Standards or test serum into each well.
2. Dispense 100µl of Conjugate into each well and mix thoroughly for 30 seconds.
3. Incubate for 60 minutes at room temperature (20ºC to 25ºC).
4. Discard well contents and wash 5 times with distilled water.
5. Add 100µl of Substrate solution to each well. Gently shake for 5 seconds.
6. Incubate in the dark for 20 minutes at room temperature (20ºC to 25ºC).
7. Add 100µl of Stop Solution to each well and gently shake for 30 seconds.
8. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450 nm filter.

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