PATHOZYME® FREE THYROXINE Ref OD467

Enzyme Immunoassay for the quantitative determination of fT4 in human serum

Store at 2°C to 8°C. DO NOT FREEZE.

For in vitro use only.

INTRODUCTION

L-Thyroxine (T4) or 3,5,3',5'-tetraiodothyronine is the most commonly measured thyroid hormone for the diagnosis of thyroid function. T4 has its primary influence on protein synthesis and oxygen consumption in virtually all tissues but it is also important for growth, development, and sexual maturation.

T4 is synthesised by the thyroid gland and is secreted into the bloodstream. Here the T4 becomes bound to serum proteins for transport to the cells. The major transport protein is Thyroxine Binding Globulin (TBG) which normally accounts for 80% of the bound T4. Other thyroid hormone binding proteins are Thyroxine Binding Prealbumin and Albumin. Most of the serum T4 is bound to these transport proteins leaving only about 0.03% free to exert its effect on cells. It is the free T4 (fT4) that represents the metabolically active fraction; for this reason the measurement of fT4 concentration is considered to be an indicator of patient thyroid status.

Primary hypothyroidism results in underproduction of T4 by the thyroid gland and consequently an abnormally low circulating fT4 concentration is in the blood. Primary hyperthyroidism leads to excessive thyroid production on T4 and resulting elevated fT4 concentration.

Total serum T4 concentrations are dependent on the level of circulating TBG as well as the patient's thyroid status. The concentration of TBG can be affected by certain drugs, steroid hormones, pregnancy, and by various nonthyroid illnesses. In an earlier generation of thyroid function tests, the effect of variable TBG concentration was dealt with by calculating a Free Thyroxine Index (FTI). This FTI is the product of Total T4 concentration and Thyroid Uptake (TU), which assesses the number of available binding sites on the TBG. This approach requires carrying out two separate assay determinations (total T4 and TU), but does provide a better indicator of thyroid status than total T4 alone.

fT4 tests are designed to directly reflect the equilibrium existing in serum between T4 and TGB-bound T4. These methods, including the fT4 tests, can generally reflect thyroid status in a single assay.

INTENDED USE

PATHOZYME FREE T4 is an Enzyme Immunoassay (EIA) for the quantitative determination of Free Thyroxine (fT4) in human serum.

For professional use only.

PRINCIPLE OF THE TEST

The fT4 test is a solid phase competitive enzyme immunoassay. Patient serum samples, standards, and Thyroxine-Enzyme Conjugate are added to wells coated with monoclonal T4 antibody. After incubation at room temperature, the wells are washed to remove unbound T4 conjugate. On the addition of Substrate (TMB), a colour develops only in those wells in which enzyme is present, indicating a lack of fT4. The reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450 nm. The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabelled fT4 in the sample.

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

CONTENTS

Ref
OD467

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 T4.
Ready to use. (Colourless)

Cal B Level as stated on vial — **1ml**
Reference Standard: Free T4 diluted in human serum.
Ready to use. (Colourless)

Cal C Level as stated on vial — **1ml**
Reference Standard: Free T4 diluted in human serum.
Ready to use (Colourless)

Cal D Level as stated on vial — **1ml**
Reference Standard: Free T4 diluted in human serum.
Ready to use (Colourless)

Cal E Level as stated on vial — **1ml**
Reference Standard: Free T4 diluted in human serum.
Ready to use (Colourless)

Cal F Level as stated on vial — **1ml**
Reference Standard: Free T4 diluted in human serum.
Ready to use (Colourless)

Conj — **10.5ml**
T4 HRP Conjugate: T4 conjugated to Horseradish Peroxidase.
Ready to use. (green)

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

PATHOZYME FREE T4 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 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 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 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 the standards and test serum into the assigned wells.
5. Add 100µl of Thyroxine Enzyme Conjugate to all wells. Swirl the microplate gently for 30 seconds to mix.
6. Incubate for 60 minutes at room temperature (20°C to 25°C).
7. 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. 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. 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. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
11. Add 100µl of Substrate to all wells.
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. It is important to make sure that all the blue colour changes completely to a yellow colour.
15. 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 control serum 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 samples. Construct a standard curve by plotting the mean absorbance obtained for 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 fT4 in pg/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.
Based on random selected out-patient clinical laboratory samples, the normal range of fT4 is 8-22 pg/ml. The minimum detectable concentration of fT4 by **PATHOZYME FREE T4** is estimated to be 0.5 pg/ml.

Substance	Cross Reactivity (%) at 100µg/ml Thyroxine equivalent
d - Triiodothyronine	0.0150
l - Triiodothyronine	0.0300
Iodothyrosine	0.0001
Diiodothyrosine	0.0001
Diiodothyronine	0.0001

EVALUATION DATA

Calibrated to major competitors and in house standards.
The co-efficient of variation of **PATHOZYME FREE T4** is less than or equal to 10%.

In an evaluation between the Omega Pathozyme Free T4 kit and a coated tube radioimmunoassay analogue method for samples with levels between 1 and 80 pg/ml the following data was generated.

Number of Samples	85
Correlation Co-efficient	0.978
Slope	0.952
Intercept	0.1
Omega Mean	15 pg/ml
Radioimmunoassay Mean	14 pg/ml

These kits were shown to give good correlation.

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QUICK REFERENCE TEST PROCEDURE

1. Dispense 50µl of Standards or test serum into each well.
2. Dispense 100µl of Thyroxine Enzyme 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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OMEGA DIAGNOSTICS LTD.
Omega House, Hillfoots Business Village
Alva FK12 5DQ, Scotland, United Kingdom
odl@omegadiagnostics.co.uk
www.omegadiagnostics.com
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