

PATHOZYME[®] TOTAL TRIIODOTHYRONINE ^{Ref} OD367

Enzyme-Immunoassay (EIA) for the detection of T3 in human serum

Store at 2°C to 8°C. DO NOT FREEZE

For in-vitro use only

INTRODUCTION

Triiodothyronine (3, 5, 3triiodo-L-tyronine, T3) and Thyroxine (T4) are two active hormones found in the bloodstream. Approximately 20% of circulating T3 is derived from direct synthesis and secretion by the thyroid gland, while 80% is produced by the deiodination of T4 in peripheral tissues. T3 is transported through the peripheral bloodstream primarily bound to serum proteins, specifically Thyroxine Binding Globulin (TBG), Thyroid Binding Prealbumin (TBP) and Albumin. Only about 0.3% of the total serum T3 is unbound and free to diffuse into tissues to exert its biological effects. T3 has its primary influence on the rate of oxygen consumption and heat production in almost all tissues. The hormone also plays a critical role in the growth, development and sexual maturation of growing mammals.

Total serum T3 is one parameter used in the differentiation and clinical diagnosis of thyroid disease, in particular Hyperthyroidism. In most hyperthyroid patients both T3 and T4 are elevated. Approximately 5-10% of all cases of hyperthyroidism, however, have elevated T3 concentrations accompanied by normal T4 concentrations which is known as T3-thyrotoxicosis. Such clinical conditions make it vital to establish that T3 levels are normal before excluding the diagnosis of hyperthyroidism. Serum T3 levels are also an excellent indicator of the ability of the thyroid to respond to stimulation and suppression test.

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

INTENDED USE

PATHOZYME T3 is an Enzyme Immunoassay (EIA) for the quantitative determination of triiodothyronine (T3) in human serum.

For professional use only.

PRINCIPLE OF THE TEST

Goat Anti-Mouse IgG Antibody is coated onto microtitration wells. Test sera are applied along with Antibody Reagent. T3 enzyme Conjugate is added which competes with the serum T3 for available binding sites on the solid phase. After incubation, the wells are washed to remove any unbound T3 or T3 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 T3. 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

^{Ref}
OD367



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 ng/ml	1ml
Reference Standard: Human serum free of T3. Ready to use. (Colourless)	
Cal B 0.75ng/ml	1ml
Reference Standard: T3 diluted in human serum. Ready to use. (Colourless)	
Cal C 1.5ng/ml	1ml
Reference Standard: T3 diluted in human serum. Ready to use. (Colourless)	
Cal D 3.0ng/ml	1ml
Reference Standard: T3 diluted in human serum. Ready to use. (Colourless)	
Cal E 6.0ng/ml	1ml
Reference Standard: T3 diluted in human serum. Ready to use. (Colourless)	
Cal F 10 ng/ml	1ml
Reference Standard: T3 diluted in human serum. Ready to use. (Colourless)	
Washbuf 20 X	50ml
Wash Buffer concentrate: Tris based buffer containing detergents. (Colourless)	
Ab REAG	7ml
Mouse anti T3 antibody. Ready to use (Pink)	
Conj 11 X	1.3 ml
T3 HRP Conjugate concentrate: T3 conjugated to Horseradish Peroxidase. (Colourless)	
DIL Conj	12 ml
Conjugate Diluent: Phosphate based buffer containing stabilising proteins. Working Strength. (Green)	
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: 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 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 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 T3 Stop Solution is dilute Hydrochloric acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

PATHOZYME T3 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.

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

- Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assay.
- 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.
- 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.
- Dispense 50 l of Standards and test serum into the assigned wells.
- Dispense 50 l of Antibody Reagent into each well.
- Thoroughly mix for 30 seconds. It is very important to mix completely.
- Dispense 100 l of working strength conjugate into each well. Mix thoroughly for 30 seconds.
- Incubate for 60 minutes at room temperature (20°C to 25°C).

9. 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.
10. 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.
11. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
12. 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.
13. Dispense 100 l Substrate Solution into each well and mix gently for 5 seconds.
14. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
15. Stop the reaction by adding 100 l Stop Solution to each well.
16. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
17. 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 T3 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.

Based on random selected out-patient clinical laboratory samples, the normal range of T3 is 0.8-1.9ng/ml. The minimum detectable concentration of T3 by **PATHOZYME T3** is estimated to be 0.2ng/ml.

EVALUATION DATA

Calibrated to major competitors and in house standards.

The co-efficient of variation of **PATHOZYME T3** is less than or equal to 10%.

In an evaluation between the Omega **Pathozyme T3** kit and the Abbott AxSym Total T3 Kit for samples with levels between 0.32 and 5.9 ng/ml the following data was generated.

Number of Samples	67
Correlation Co-efficient	0.906
Slope	0.920
Intercept	- 0.229
Omega Mean	1.24 ng/ml
Abbott Mean	0.91 ng/ml

In an evaluation between the Omega **Pathozyme T3** kit and the Monobind Total T3 Kit for samples with levels between 0.14 and 6.2 ng/ml the following data was generated.

Number of Samples	80
Correlation Co-efficient	0.993
Slope	1.004
Intercept	- 0.084
Omega Mean	1.21 ng/ml
Monobind Mean	1.13 ng/ml

In both studies the kits gave good correlation.

REFERENCES

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QUICK REFERENCE TEST PROCEDURE

1. Dispense 50 l of Test Serum, Controls or Standards.
2. Dispense 50 l of Antibody Reagent into each well and mix thoroughly for 30 seconds.
3. Dispense 100 l of working strength conjugate into each well and mix thoroughly for 30 seconds.
4. Incubate for 60 minutes at room temperature (20°C to 25°C).
5. Discard well contents and wash five times with wash buffer.
6. Add 100 l of Substrate Solution to each well. Gently shake for 5 seconds.
7. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
8. Add 100 l of Stop Solution to each well and gently shake for 30 seconds.
9. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

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