Free T3 (Free Triiodothyronine) Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAFT31	Free T3 Elisa	96 Tests

Intended Use:
Free T3 Elisa is intended for quantitative measurement of Free Triiodothyronine in human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:
Free Triiodothyronine represents 0.25% of the Total T3 and is the physiologically active fraction. Measurement of FT3 is very important for patients with symptoms of Hyperthyroidism, Grave's disease and Goiter.

The FT3 assay is based on a one step competitive method. The sample, T3 coated microwells and enzyme labelled Anti T3 are combined in the reaction. During the incubation, T3 coated on microwells, the FT3 present in the sample, compete for binding sites in the enzyme labelled antibodies. After washing and addition of substrate solution, there results a chromogenic reaction which is stopped by the addition of a stopping solution. This results in a final yellow coloured solution which is measured in a Elisa Reader. The colour intensity is inversely proportional to the amount of FT3 in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with T3 analogue. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 2 months at 2-8°C.
FT3 Calibrators	6x1ml	6 vials containing T3 at concentrations of 0.0, 2.0, 5, 10, 25 and 50 pmol/l (1pmol/l x 0.651 = 1 pg/ml) made up in a human serum matrix in PBS with preservatives. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable for 1 month at 2-8°C.
Enzymatic Conjugate	1x6ml	1 vial containing 6ml of HRP labelled sheep monoclonal Anti T3 antibodies in Buffered saline containing BSA. Contains 0.2% Proclin 300. Once open, stable for 2 months at 2-8°C.
Wash Buffer Concentrate (40X)	1x25ml	PBS-Tween at pH 7.4. 40X concentrate. The concentrate must be diluted 1 to 40 with distilled water before use. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x11ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable for two months at 2-8°C.
Stop Solution	1x6ml	Diluted Sulfuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2-8°C.

Traceability: The calibrators are matched to a working calibrator. The working calibrator is manufactured by gravimetric methods by the addition of T3 antigen to hormone free human serum.
Also Provided: Plastic Sealable bag, IFU and Cardboard plate covers.

Materials required but not provided:
Automatic microplate reader, Microplate washer, distilled water, plate shaker, micropipettes, incubator, disposable reagent troughs.

Specimen Collection:
- Collect serum samples in accordance with correct medical practices.
- Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 7 days at 2-8 °C, and 1 month at -20 °C. Recovery within 90-110 % of serum value or slope 0.9-1.1. Freeze only once.
- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
- Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
- Avoid grossly hemolytic, lipemic or turbid samples.
- Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
- If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.

Storage and Stability:
1. Store all components at 2-8°C. Do not freeze. Avoid strong light.
2. Place unused wells in the zip-lock bag with desiccant provided, the seal the zip-lock bag in the aluminium foiled pouch with a plate lid and return to 2-8°C, under which conditions the wells will remain stable for 2 months, or until the labelled expiry date, whichever is earlier.
3. Seal and return unused calibrators to 2-8°C, under which conditions the stability will be retained for 1 months, for longer use, store opened calibrators in aliquots and freeze at -20°C. Avoid multiple freeze thaw cycles.
4. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the expiry date whichever is earlier.

Precautions and Safety:
The Elisa assays are time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure and do not modify them.

1. **For professional use only.**
2. **Follow the instructions in this IFU as reliability of results cannot be guaranteed if there are deviations from the instructions.**
3. **The calibrators contain human serum based components. They have been tested and found to be non-reactive to HBsAg, HIV and HCV antibodies and syphilis. The assay contains materials of animal origin like BSA which have been sourced from countries where BSE has not been reported. It is recommended that all human serum based material may be considered potentially infectious and care to be taken in their use.**
4. **Wear laboratory protection equipment such as gloves, glasses whilst handling reagents, controls and samples. Wash hands thoroughly after each operation.**
5. **Samples in the microwells should not have bubbles as these bubbles may result in erroneous results.**
6. **Wash the wells completely. Avoid overflow during wash. Remove any residual wash buffer by tapping the microwells on a clean towel or absorbent paper. It is ideal to use an automated microplate washer.**
7. **Use new pipette tips for each pipetting to avoid cross contamination.**
8. **Do not use kits after expiry date**
9. **Do not interchange components from other kits.**
10. **The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore the substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.**
11. **If more than one plate is used, it is recommended that the calibration curve is repeated.**
12. **Secure the calibrator vial caps, if unused calibrators are stored for further use.**
13. **It is important that the time of reaction in each swell is held constant to achieve reproducible results.**
14. **It is important to calibrate all equipment e.g. micropipettes, microplate readers, automated microplate strip washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.**
15. **Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.**
16. **Failure to remove adhering solution adequately in the washing step will lead to erroneous results.**
17. **Use no more than 32 wells for each assay run, when manual pipette is used. Complete pipetting of all calibrators, controls and samples within 5 minutes.**

Procedure:
Reagent preparation:
Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use. Adjust the incubator to 37 °C. Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature. Do not use Substrate if it looks blue. Do not use reagents that are contaminated or have bacteria growth.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators: Add 50ul of Calibrators and Samples to each well.

STEP 3

Addition of Enzyme Conjugate: Add 50ul of Enzyme Conjugate solution to each well. Shake the plate for 30 seconds to ensure that the added components are well mixed.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer of 350ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of the Substrate: Add 100ul of Substrate Solution to each well.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 50ul of the Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

STEP 9

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader. Note down the absorbances. The results should be read within 30 minutes of adding the stop solution.

Calculation of results:
- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in pmol/l in X axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

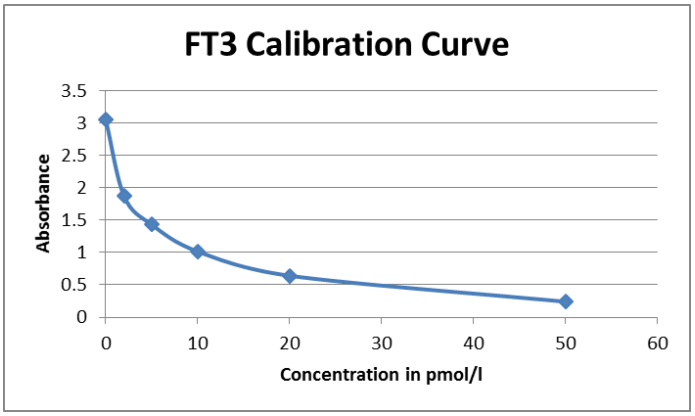
The following data is for demonstration only and cannot be used in place of data generations at the time of assay:

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	3.057	0 pmol/l
CAL B	1.871	2 pmol/l

Prestige Diagnostics U.K. Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100
www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

CAL C	1.431	5 pmol/l
CAL D	1.016	10 pmol/l
CAL E	0.642	20 pmol/l
CAL F	0.240	50 pmol/l
Control Level 1	1.456	4.83 pmol/l
Control Level 2	0.876	15.61 pmol/l
Sample	1.396	5.42 pmol/l



Quality Control:

Each laboratory should establish assay controls at levels in the low, normal, and elevated range for monitoring assay performance. There controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.
It is recommended that each test run should be accompanied with quality controls. Recommended controls are QCCIAL1, QCCIAL2, QCCIAL3 – Immunoassay Controls L1, L2 and L3.

Limitations - interference

• The assay is unaffected by icterus (bilirubin < 600 µmol/L or < 35 mg/dL), hemolysis (Hb < 0.559 mmol/L or < 0.9 g/dL), lipemia (Intralipid < 1200 mg/dL), and biotin < 94 nmol/L or < 23 ng/mL.
• Criterion: Recovery within ± 10 % of initial value.
• Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
• Performance of this test has not been established with neonatal samples.
• In severe NTI (nonthyroidal illness), the assessment of thyroid status becomes very difficult. TSH measurements are recommended to identify thyroid dysfunction.
• Familial dysalbuminemic conditions may yield erroneous results on direct free T3 assays.
• If a patient, for some reason, reads higher than the highest calibrator report as such (e.g. > 50 pmol/l).
Do not try to dilute the sample. TBG variations in different matrices will not allow FT3 hormones to dilute serially.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L, pg/mL or ng/dL).
Conversion factors:
pmol/L x 0.651 = pg/mL
pg/mL x 1.536 = pmol/L
pg/mL x 0.1 = ng/dL

Limits and ranges

Measuring range
0.5-50.0 pmol/L or 0.325-32.55 pg/mL(defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.5 pmol/L or < 0.325 pg/mL. Values above the measuring range are reported as >50.0 pmol/L or 32.55 pg/mL.

Lower limits of measurement
Lower detection limit
Lower detection limit: 0.5 pmol/L or 0.325 pg/mL.
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Expected values
3.3-7.5 pmol/L
These values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 851 healthy test subjects examined.
We have not studied the reference intervals in children, adolescents and pregnant women. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n= 40). The following results were obtained:

		Repeatability*		Intermediate precision	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human Serum 1	5.77	0.39	6.84	0.50	8.72
Human Serum 2	13.86	0.79	5.71	1.03	7.46
Human Serum 3	28.6	1.56	5.46	2.09	7.31
PC Universal 1	4.46	0.31	7.02	0.31	6.88
PC Universal 2	12.15	0.81	6.66	0.84	6.93

*Repeatability = within-run precision

Method comparison
A comparison of the FT3 assay (y) with the Roche Cobas FT3 (x) using clinical samples gave the following correlations:
Number of samples measured: 121

Linear regression
y = 1.0407x - 0.2867
r = 0.9768
The sample concentrations were between approx. 2.5 and 40pmol/L.
Analytical specificity
For the antibody derivative used, the following cross-reactivities were found: D-T3 100 %; L-T4 < 0.31 %; D-T4 < 0.45 %; L-rT3 <0.05 %; L-T2 < 0.8 %.

Limitations:

- The assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
- If the results are inconsistent with clinical findings, additional testing is suggested to confirm the results.
- Heterophilic antibodies and RF in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed.
- This assay is not suitable for neonatal samples.
- In Non-thyroidal illnesses, the assessment of thyroid status is carried out using a variety of assays. TSH is also recommended to be performed.
- Serum FT3 concentration may be elevated under conditions such as pregnancy or administration of Oral contraceptives.
- Samples with values higher than the highest calibrator must be reported as >50 pmol/l. Do not dilute the samples. TBG variations in different matrices will not allow FT3 to dilute serially.
- Familial dysalbuminemic conditions may yield erroneous results on direct Free T3 assays.

References:

1. Piketty M, d' Herbomez M, Le Guillouzic D, et al. Clinical comparison of three labeled-antibody immunoassays of free triiodothyronine. *Clin Chem*. 1996;42(6):933-941.
2. Wilkins T, Midgley J, Stevens R, Caughey I, Barron N. Assay performance and tracer properties for two analog-based assays of free triiodothyronine. *Clin Chem*. 1986;32(3):465-469.
3. Bartalena L, Robbins J. Variations in Thyroid Hormone Transport Proteins and Their Clinical Implications. *Thyroid*. 1992;2(3):237-245.
4. Carrero JJ, Qureshi AR, Axelsson J, et al. Clinical and biochemical implications of low thyroid hormone levels (total and free forms) in euthyroid patients with chronic kidney disease. *J. Intern. Med*. 2007;262(6):690-701.
5. Verheecke P. Free triiodothyronine concentration in serum of 1050 euthyroid children is inversely related to their age. *Clin Chem*. 1997;43(6):963-967.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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