FT3

Free Triiodothyronine (ELISA)

diasino

REF: DS177704 Σ 96

Intended use
The DiaSino FT3 assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative determination of free triiodothyronine (FT3) in human serum. The assay is useful in the diagnosis and treatment of thyroid disorders. For professional use only.

Summary
Triiodothyronine is one of the thyroid hormones present in serum which regulates metabolism. Determination of this hormone concentration is important for the diagnostic differentiation of euthyroid, hyperthyroid, and hypothyroid states. The major fraction of total triiodothyronine is bound to the transport proteins (TBG, prealbumin, albumin). Free triiodothyronine (FT3) is the physiologically active form of the thyroid hormone triiodothyronine (T3). The determination of free T3 has the advantage of being independent of changes in the concentrations and binding properties of the binding proteins; additional determination of a binding parameter (T-uptake, TBG) is therefore unnecessary.[1,2,3]
In normal thyroid function, as the concentrations of the carrier proteins alter, the total T3 level changes so that the FT3 concentration remains constant.[4] Thus, measurements of FT3 concentrations correlate more reliably with clinical status than total T3 levels. For example, the increase in total T3 levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T3 levels while the FT3 concentration remains basically unchanged.[5] In addition, it has been found that the mean FT3 value has a gradient decreasing from young to older.[6]

Test principle
Competition principle. Total duration of assay: **80 minutes**
- Sample, T3 derivant coated microwells and enzyme labeled Anti-T3 are combined.
- During the incubation, T3 derivant coated on microwells and FT3 present in the sample compete for binding to the enzyme labeled antibodies.
- After washing, a complex is generated between the solid phase and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The color intensity is inversely proportional to the amount of FT3 in the sample.

Reagents
Materials provided
- **FT3 Coated Microplate - symbol** FT3 PLATE 8 x 12 strips, 96 wells, pre-coated with T3 derivant.
- **FT3 Calibrators - symbols** FT3 CAL A-F 6 vials, 1 mL each, ready to use; Concentrations: 0(**A**), 2(**B**), 5(**C**), 10(**D**), 20(**E**) and 50(**F**) pmol/L.
- **FT3 Enzyme Conjugate - symbol** FT3 CONJ 1 vial, 6.0 mL of HRP (horseradish peroxidase) labeled sheep monoclonal Anti-T3 in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.2% ProClin300 preservative.
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU -** 1 copy.
- **Plate Lid**: 1 piece.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05%-0.2% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Incident report
Any suspected serious incidents related to this assay shall be immediately reported to DiaSino, DiaSino's Authorized Representative in the EU, and the national competent authorities of the Member States where the users and/or patients are located.

Storage and stability
- Store at 2-8℃.
- Seal and return unused reagents to 2-8℃, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum is recommended for this assay.
- Cap and store the samples at 18-25 ℃ for no more than 8 hours. Stable for 7 days at 2-8℃, and 1 month at -20℃. Freeze only once.
- Do not use heat-inactivated samples.
- 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.
- Avoid grossly hemolytic, lipemic or turbid samples.

Calibration
The DiaSino T3 ELISA has been standardized against the Elecsys FT3 assay which was standardized using equilibrium dialysis.[7,8]
Recalibration is recommended when a new reagent lot is used, or the quality controls are out of specified range.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The 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.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate.
Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure
Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 ℃) before measurement. Mix all reagents through gently inverting prior to use.
- Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
- Add **50 µL** of **calibrators** or **samples** to each well.
- Add **50 µL** of **enzyme conjugate** to each well.
- Shake the microplate gently for **30 seconds** to mix.
- Cover the plate with a plate lid and incubate at **37 ℃** for **60 minutes**.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add **350 µL** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of 5 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 µL** of **substrate** to each well.
- Cover and incubate at **ambient temperature (18-25℃)** in the dark for reaction for **20 minutes**. Do not shake the plate after substate addition.
- Add **50 µL** of **stop solution** to each well.
- Shake for **15-20 seconds** to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
- Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

Calculation
- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in pmol/L for each calibrator.
- Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

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

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Sample	Value (pmol/L)	Absorbance
Calibrator A	0	3.057
Calibrator B	2	1.871
Calibrator C	5	1.431
Calibrator D	10	1.016
Calibrator E	20	0.642
Calibrator F	50	0.240
Control 1	4.83	1.456
Control 2	15.61	0.876
Sample	5.42	1.396

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.
- 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

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 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 DiaSino 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 DiaSino FT3 assay (y) with the Roche Elecsys FT3 (x) using 89 clinical samples gave the following correlations:
Linear regression
y = 1.0407x - 0.2867
r = 0.9768

The sample concentrations were between approx. 2.5 and 40 pmol/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 %.

Functional sensitivity

0.55 pmol/L or 0.358 pg/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

References

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Symbols

IVD In vitro diagnostic medical device	8°C / 2°C Temperature limit	Consult instructions for use	REF Catalog number
LOT Batch code	Date of manufacture	Use-by date	Σ Contains sufficient for <n> tests
Manufacturer	Do not use if package is damaged and consult instructions for use	CE European Conformity	EC REP Authorized representative in the European Community

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