

T4

Thyroxine (ELISA)

REF: DS177703



Intended use

The DiaSino T4 assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative determination of total thyroxine (T4) in human serum. The assay is useful in the diagnosis and treatment of thyroid disorders. For professional use only.

Summary

The hormone thyroxine (T4) is the main product secreted by the thyroid gland and is an integral component of the hypothalamus-anterior pituitary-thyroid regulating system. It has the function of anabolically influencing metabolism. Thyroxine is formed in a coupling reaction from two DIT molecules (3,5-diiodotyrosine) in the thyroid gland. It is stored bound to thyroglobulin in the lumina of the thyroid follicles and is secreted as required under the influence of TSH.^{1,2} The major part (> 99 %) of total thyroxine (T4) in serum is present in protein-bound form. As the concentrations of the transport proteins in serum are subject to exogenous and endogenous effects, the status of the binding proteins must also be taken into account in the assessment of the thyroid hormone concentration in serum. If this is ignored, changes in the binding proteins (e.g. due to estrogen-containing preparations, during pregnancy or in the presence of a nephrotic syndrome etc.) can lead to erroneous assessments of the thyroid metabolic state.^{3,4,5,6,7}

The determination of T4 can be utilized for the following indications: the detection of hyperthyroidism, the detection of primary and secondary hypothyroidism, and the monitoring of TSH-suppression therapy.⁸

The DiaSino T4 assay employs a competitive test principle with an antibody specifically directed against T4. Endogenous T4, released by the action of 8-anilino-1-naphthalene sulfonic acid (ANS).

Test principle

Competition principle. Total duration of assay: **80 minutes**.

- Sample, T4 derivant coated microwells and enzyme labeled Anti-T4 are combined.
- During the incubation, T4 derivant coated on microwells and T4 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 T4 in the sample.

Reagents

Materials provided

- **T4 Coated Microplate** - symbol **T4 PLATE** 8 x 12 strips, 96 wells, pre-coated with T4 derivant.
- **T4 Calibrators** - symbols **T4 CAL A-F** 6 vials, 1 mL each, ready to use; Concentrations: 0(A), 2.5(B), 5(C), 10(D), 15(E) and 30(F) µg/dL.
- **T4 Enzyme Conjugate** - symbol **T4 CONJ** 1 vial, 6.0 mL of HRP (horseradish peroxidase) labeled mouse monoclonal T4 in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.2% ProClin300 preservative. ANS 1.5 mg/mL.
- **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°C.
- Seal and return unused reagents to 2-8°C, 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 °C for no more than 8 hours. Stable for 7 days at 2-8°C, and 1 month at -20°C. 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 T4 ELISA has been standardized against the the Elecsys T4 assay which has been checked by ID-GC/MS (isotope dilution gas chromatography mass spectrometry) on various control materials.⁹ 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 °C) 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 °C 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°C)** in the dark for reaction for **20 minutes**. Do not shake the plate after substrate 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 µg/dL 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.

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

Sample	Value (µg/dL)	Absorbance
Calibrator A	0	3.077
Calibrator B	2.5	2.172
Calibrator C	5	1.414
Calibrator D	10	0.777
Calibrator E	15	0.334
Calibrator F	30	0.191
Control 1	8.7	0.943
Control 2	14.41	0.386
Sample	9.32	0.863

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.
- Serum T4 concentration is dependent upon a multiplicity of factors: hypothalamus gland function and its regulation, TBG concentration, and the binding of T4 to TBG. Thus, total T3 concentration alone is not sufficient to assess clinical status.
- Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L, µg/dL or µg/L).

Conversion factors:

nmol/L x 0.077688 = µg/dL

µg/dL x 12.872 = nmol/L

nmol/L x 0.77688 = µg/L

Measuring range

0.50-30 µg/dL or 6.43-385.80 nmol/L (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as <0.5 µg/dL or <6.43 nmol/L. Values above the measuring range are reported as >30 µg/dL or >385.80 nmol/L.

Lower detection limit

0.50 µg/dL or 6.43 nmol/L

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

4.5-13.8 µg/dL or 57.9-177.6 nmol/L

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:

Sample	Mean µg/dL	Repeatability*		Intermediate precision	
		SD µg/dL	CV %	SD µg/dL	CV %
Human Serum 1	3.94	0.36	9.14	0.35	8.88
Human Serum 2	9.27	0.51	5.50	0.68	7.32
Human Serum 3	15.84	0.72	4.55	0.79	4.99
PC Universal 1	7.33	0.32	4.36	0.27	3.68
PC Universal 2	15.64	0.57	3.64	0.85	5.43

*Repeatability = within-run precision

Method comparison

A comparison of the DiaSino T4 assay (y) with the Elecsys T4 (x) using 91 clinical samples gave the following correlations:

Linear regression

y = 1.0296x - 1.2318

r = 0.9689

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The sample concentrations were between approx. 1 and 25 µg/dL.

Analytical specificity

For the antibody derivative used, the following cross-reactivities were found: L-T4 and D-T4 100 %; L-T3 1.89 %; D-T3 1.44 %; 3-iodo-L-tyrosine 0.002 %; 3,5-diiodo-L-tyrosine 0.008 %.

Functional sensitivity













0.58 µg/dL or 7.47 nmol/L

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

 In vitro diagnostic medical device	 Temperature limit	 Consult instructions for use	 Catalog number
 Batch code	 Date of manufacture	 Use-by date	 Contains sufficient for <n> tests
 Manufacturer	 Do not use if package is damaged and consult instructions for use	 European Conformity	 Authorized representative in the European Community



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