TRUSTwell®

Total Triiodothyronine (T3) ELISA Kit

IVD | REF AE1010

- 96-well ELISA kit for the quantitative determination of Total Triiodothyronine (T3) concentration in human serum
- Store at 2-8°C upon receipt

INTENDED USE

The Total Triiodothyronine (T3) ELISA is a competitive solid-phase enzyme-linked immunosorbent assay for the quantitative determination of T3 concentration in human serum. It is intended to be used by professionals as an aid in the diagnosis of thyroid dysfunction.

INTRODUCTION

Thyroid disease is very common on a global level and studies have shown that the prevalence of undiagnosed thyroid disorder is high[1-3]. Thus, quantification of markers that reflect thyroid function is very important. Thyroid function and metabolism are controlled by the human hypothalamic-pituitary-thyroid (HPT) axis, which is made up of the hypothalamus, the pituitary gland, and the thyroid. Thyroid stimulating hormone (TSH) controls the production of the thyroid hormones thyroxine (T4) and 3, 5, 3' triiodothyronine (T3), which play an important role in the regulation of metabolism[4-6].

The majority of T3 and T4 that circulates in the blood is protein-bound, and attaches to plasma proteins such as Thyroxine-Binding Globulin (TBG). The small percentage that is not protein-bound is considered "free" (fT4/fT3), and can readily enter target tissue. Importantly, the binding protein concentration of each individual can be affected by factors such as pregnancy, hormonal therapy, and certain medications, which will, therefore, affect T4/T3 test results[6-8]. Thus, testing of thyroid function commonly encompasses TSH and a combination of the thyroid hormones (T3/T4, fT3/fT4) assays[3,7,8].

Total T3 tests can be used to aid in the detection and diagnosis of thyroid disease including hyperthyroidism, hypothyroidism, and Graves' disease, and to monitor pregnancy and treatment for thyroid disorder, pituitary disease, and thyroid cancer[8,9]. T3 measurements are generally employed in conjunction with results from other members of the thyroid function panel as well as a measurement or estimate of binding protein levels such as TBG to give the most accurate measurement of thyroid status possible[7].

TEST PRINCIPLE

The Total Triiodothyronine (T3) ELISA is a competitive solid-phase enzyme-linked immunosorbent assay for the quantitative measurement of T3 concentration in human serum.

The Total Triiodothyronine (T3) ELISA is comprised of three key components:
1) Solid microwells pre-coated with anti-T3 antibody,
2) T3 Calibrators,
3) T3 Enzyme Concentrate (11X) comprised of horseradish peroxidase conjugated to T3 (T3-HRP)*
   *Note: Reagent preparation required: T3 Enzyme Concentrate (11X) will be diluted with Enzyme Buffer to result in T3 Enzyme Reagent

During the assay, the calibrator, control, or test specimen as well as the T3-HRP are added to the T3-antibody coated microwell. During the 60 minute incubation, the T3 in the test specimen and in the T3-HRP compete to bind the antibody coated on the microwell surface.

Unbound material is then removed by washing. In the next step, the TMB Substrate is added and the presence of the HRP bound to microwell surface is shown by the development of a blue color. The reaction is then terminated with the Stop Solution and the optical density (OD) is determined using a spectrophotometer at 450/620-690 nm. The color intensity reflects the amount of T3-HRP bound to the microwell surface, and is inversely proportional to the amount of T3 in the control or test specimen.

A standard curve can then be developed by plotting the T3 Calibrator concentrations on the x-axis against the relative OD values on the y-axis. The T3 concentration of each assay control or test specimen can then be interpolated from the standard curve.

MATERIALS AND REAGENTS

Materials and reagents provided:

Item	Description	Quantity	Catalog
1	Anti-T3 Ab Coated Microwells	8 wells x 12 strips	AE1010W
2	Total T3 Calibrators: C1 (0 ng/mL)	1.0 mL	AE1010C1
3	C2 (0.5 ng/mL)	1.0 mL	AE1010C2
4	C3 (1.0 ng/mL)	1.0 mL	AE1010C3
5	C4 (2.0 ng/mL)	1.0 mL	AE1010C4
6	C5 (4.0 ng/mL)	1.0 mL	AE1010C5
7	C6 (8.0 ng/mL)	1.0 mL	AE1010C6
8	Total T3 Assay Control	1 mL	AE1010AC
9	Total T3 Enzyme Concentrate (11X)	1.5 mL	AE1010EC
10	Enzyme Buffer	12 mL	AE1010EB
11	Wash Buffer Concentrate (40X)	20 mL	AWE3002
12	TMB Substrate	12 mL	ATME2000
13	Stop Solution	7 mL	ASE1000
14	Product Insert	1	PI-AE1010
15	ELISA Working Sheet	2	AE0001ES
16	Microplate Sealers	3	
17	Desiccant	4	

**The Assay Control values are lot-specific. Please see vial labels for exact concentration ranges.*

Materials and reagents required but not provided in the kit
1. Pipette capable of delivering 50 µL, 100 µL, and 1 mL
2. Microplate reader
3. Vortex mixer or equivalent
4. Absorbent paper for blotting the microwells
5. Graph paper
6. Timer
7. Distilled or de-ionized water

STORAGE AND STABILITY

All reagents except the concentrated wash buffer and enzyme reagent are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Ensure that the reagents are brought to room temperature before opening. After removing the desired number of wells, place unused wells in the resealable foil pouch provided with desiccant and return to 2-8°C. All reagents are stable through the expiration date printed on the label if not opened. Once opened, the kit is stable for 8 weeks at 2-8°C, or until the labeled expiration date, whichever is earlier.

SPECIMEN COLLECTION AND PREPARATION

- Serum specimens should be prepared from whole blood obtained by acceptable venipuncture technique.
- If not tested immediately, the specimens can be stored at 2-8°C for up to 7 days. The specimens should be frozen at -20°C for longer storage. Avoid multiple freeze-thaw cycles. If a specimen is to be shipped, pack in compliance with federal regulations covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation before testing.
- Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity.
- Specimens containing sodium azide may interfere with test results.

PREPARATION OF THE REAGENTS

1. Bring all reagents to room temperature (22-28°C).
2. Prepare T3 Enzyme Reagent: Dilute T3 Enzyme Concentrate (11X) in Enzyme Buffer in a ratio of 1:11. For example, for 48 wells, add 0.48 mL of the T3-HRP (11x) to 4.8 mL of the enzyme buffer. Dilute only 10-12% more than needed in required determinations. Use the formula below to determine the volumes.

   Volume of Enzyme Buffer (EBV) = Number of wells x 0.1 = ____ mL

   Volume of T3 Enzyme Conjugate (11x) = EBV x 0.1 = _____ mL
3. **Preparation of working Wash Buffer:**
   If precipitants are visible, warm up the Wash Buffer (40X concentrate) at 37°C. Dilute concentrated Wash Buffer 40-fold with water as follows:

Plate	DI water	40X wash buffer	Final volume
Full plate	195 mL	5 mL	200 mL
Half plate	97.5 mL	2.5 mL	100 mL
Quarter plate	48.75 mL	1.25 mL	50 mL

4. Mix each reagent before adding to the test wells.
5. Determine the number of strips needed and mark on the ELISA working sheet with the appropriate information. **Calibrators should be run in duplicate to ensure accuracy.**

ASSAY PROCEDURE

1. Calculate the desired number of microwells. Remove the remaining microwells and place them with desiccant into the resealable foil pouch, seal and store at 2-8°C for later use.
2. Add 50 µL of Total T3 calibrators, T3 Assay Controls, and test specimens into the assigned wells, respectively.
3. Add 100 µL of Total T3 Enzyme Reagent (*see reagent preparation*) into all the wells.
4. Cover the plate with a microplate sealer, and then shake gently for 10 seconds.
5. Incubate the microplate at room temperature (22-28°C) for 60 minutes.
6. Wash Step (Can be performed manually or with automated washing):
   Manual washing: Carefully remove the incubation mixture by disposing the solution into a waste container. Fill each well with 350 µL of working wash buffer and mix gently for 20-30 seconds. Discard the wash solution completely. Repeat 2 more times. After completing the last wash step, tap the plate on absorbent paper to remove residual liquid.
   Automated washing: Automatic plate washer must be calibrated to ensure efficient washing. Fill each well with 350 µL of diluted wash buffer. Aspirate all wells completely. Repeat 2 more times.
7. Add 100 µL of TMB Substrate into each well.
8. Cover the microplate and incubate at room temperature (22-28°C) for 15 minutes.
9. Stop the reaction by adding 50 µL of Stop Solution to each well. Gently mix for 10 seconds. Add the Stop Solution in the same sequence as substrate addition. **It is important to make sure that all the blue color changes completely to a yellow color.**
10. Set the microplate reader wavelength at 450 nm. Measure the OD value of each well within 30 minutes after adding Stop Solution. A filter of 620-690 nm can be used as a reference wavelength to optimize the assay result.

Flow chart of assay procedure

1.	Secure strips in microwell frame	Number of strips
2.	Add Total T3 Calibrators, Assay Controls, or specimens	50 µL
3.	Add Total T3 Enzyme Reagent	100 µL
4. 5.	Shake Incubate	10 seconds 22-28°C, 60 minutes
6.	Wash: manual or automatic	3 times
7.	Add TMB Substrate	100 µL
8.	Incubate in dark	22-28°C, 15 minutes
9.	Add Stop Solution. Gently mix	50 µL 10 seconds
10.	Read the result	450/620-690 nm within 30 minutes

CALCULATION OF RESULTS

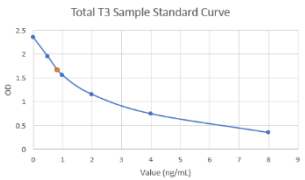
1. Calculate the mean OD value (A450/620-690) for each set of calibrators.
2. Construct a standard curve by plotting the mean OD value obtained for each calibrator against its concentration on graph paper with OD values on the vertical *y*-axis, and concentrations on the horizontal *x*-axis.
3. Interpolate concentrations for the assay controls and test specimens by plotting their mean OD values on the curve.
   Alternatively, if software is used, calculate the concentration of T3 following software menu.

4. If the OD value of a specimen is greater than that of the highest calibrator, it is recommended to dilute the specimen with C1 calibrator in a 1:1 or 1:3 ratio and test again. Any values obtained for a diluted sample must be further converted by applying the appropriate dilution factor in the calculation.

INTERPRETATION

1. Results of a typical standard curve are shown below:

Cal ID	Conc. (ng/mL)	OD
C1	0.0	2.349
C2	0.5	1.945
C3	1.0	1.552
C4	2.0	1.150
C5	4.0	0.743
C6	8.0	0.348
Patient ID	**Conc. (ng/mL)**	**OD**
1	0.82	1.700



The above data and figure are for example purposes and should not be used to calculate your result.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to check assay performance. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

The following criteria should be met to consider assay results to be valid:

- The mean OD of the '0' calibrator should be ≥ 1.3.
- CV's for duplicates of calibrators should be ≤ 15%.
- T3 Assay Control concentrations should be within the specified range on the vial labels.

NORMAL REFERENCE

1. It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local patient population and its own assay technique. The following values for the Total Triiodothyronine (T3) ELISA can be used as initial guideline ranges only:

Normal Values for T3 (ng/mL)

Sample Number (n)	75
Average	1.18
Standard Deviation (SD)	0.31
Range (± 2SD)	0.57-1.80

PEFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

Twenty (20) replicates of the '0' calibrator were run in a single assay on each of three lots of the Total Triiodothyronine (T3) ELISA, and the mean and standard deviation (SD) of the OD values were calculated. The analytical sensitivity of the test was interpolated from the standard curve. The analytical sensitivity of the Total Triiodothyronine (T3) ELISA was determined to be 0.06 ng/mL at 2SD.

2. Specificity

Specificity of the Total Triiodothyronine (T3) ELISA was evaluated by spiking potential interfering substances into a human serum matrix. Specificity was calculated by deriving a ratio between the concentration of each tested metabolite and the concentration of T3 needed to displace the same amount of conjugate. There was no cross-reactivity observed at the concentrations listed below:

d-Thyroxine	10 ug/mL
d-Triiodothyronine	100 ug/mL
Iodotyrosine	100 ug/mL
Diiodotyrosine	100 ug/mL
TBG	50 ug/mL
Human Albumin	50 mg/mL
Phenylbutazone	25 ug/mL
Phenytoin	50 ug/mL
ASA	500 ug/mL
Acetaminophen	500 ug/mL

3. Accuracy

The accuracy of the Total Triiodothyronine (T3) ELISA was determined by external comparison of 147 specimens with varying concentrations of T3 with a reference commercial ELISA. The results are listed in the following table:

Method	Mean (ng/mL)	Least Square Regression	Corr. Coef. (r)
(y)	1.89	y= 1.1558x	0.99
Reference (x)	2.08		

4. Precision

a. **Intra-assay precision:** Twenty replicates of each of three pooled human serum controls (low, medium, and high concentrations) were tested and the mean, SD, and coefficient of variation (CV) were determined. The results are shown in the following table:

Sample	N	Mean	SD	CV
Low	20	1.190	0.163	13.533
Medium	20	3.090	0.170	5.567
High	20	6.053	0.207	3.533

b. **Inter-Assay Precision:** Three human serum pooled controls (low, middle, and high concentrations) were assayed in 20 separate runs over 3 lots. The mean, SD, and CV were determined. The results are shown in the following table:

Sample	N	Mean (ng/mL)	SD	CV
Low	20	0.92	0.10	11.3%
Medium	20	2.42	0.23	9.7%
High	20	3.65	0.41	11.3%

WARNING AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the instructions in the insert will lead to inaccurate test results.
2. Do not use expired test kits.
3. Bring all reagents to room temperature (22-28°C) before use.
4. Do not use a component from any other test kit as a substitute for the components in this kit.
5. Do not use serum derived from hemolyzed blood specimens for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mouth. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. All specimens and materials used to perform this test must be disposed of as bio-hazardous waste.
10. Prior to the first incubation, and after addition of the Stop Solution, gently shake the microwells to ensure thorough mixing. Avoid splashing liquid while shaking. Do now allow formation of air bubbles in the microwell.
11. Do not allow the microwells to dry between the end of the washing operation and the reagent distribution.
12. The enzyme substrate reaction is very sensitive to metal ions. Thus, do not allow any metal elements to come into contact with the conjugate or TMB Substrate.
13. The enzyme-substrate reaction is temperature dependent. Ensure that the room temperature is between 22-28˚C during the TMB incubation.
14. The TMB substrate must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate must be stored in the dark.
15. Use a new dispensing tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate.
16. **The wash procedure is critical. Wells must be aspirated completely before adding the Wash buffer or liquid reagents. Automatic washers must be validated with the test kit prior to use. Insufficient washing will result in poor precision and falsely elevated OD values.**
17. **Microplate reader must be calibrated per manufacturer's instruction to ensure accurate OD readings. A non-calibrated reader may lead to invalid test results.**
18. Avoid exposure to strong light during color development.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Results must be followed closely when testing for T3 concentration in human specimens. Failure to follow the procedure may lead to inaccurate results.
2. The test is limited to the detection of T3 concentration in human serum.
3. A clinical diagnosis should not be based on the results of a single test and should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. Normal thyroid hormone levels do not exclude thyroid disease.
5. A variety of causes apart from thyroid dysfunction may give rise to abnormal T3 concentrations in serum.
6. Changes in circulating binding proteins (e.g. TBG) can result in altered T3 and T4 concentrations. TBG concentrations and/or binding properties can be altered by abnormal hormone levels, anabolic steroids, heparin therapy, pregnancy, phenytoin, salicylate, and other drugs.
7. If the OD value of a specimen is greater than that of the highest calibrator, it is recommended to dilute the specimen with T3 Calibrator C1 and test again.
8. Any interpretation or use of this test result must also integrate other clinical findings as well as on the professional judgment of health care providers.

REFERENCES

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Index of Symbols

Consult instructions for use	REF Catalogue number		
IVD For *in vitro* diagnostic use only	LOT Batch code		Use-by date
2 °C – 8 °C Temperature limit 2-8 °C	Do not re-use		Tests per kit N
Manufacturer	Date of manufacture		
NON STERILE If device is non-sterile	Warnings / Precautions		

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