CE **RecombiLISA**

Total Triiodothyronine (T3) ELISA

IVD | REF E1010

- 96-well ELISA kit for the quantitative determination of Total T3 concentration in human serum
- For export only, not for re-sale in the USA
- Store at 2°C-8°C upon receipt

INTENDED USE

The *RecombiLISA* Total Triiodothyronine (T3) ELISA is a competitive solid-phase enzyme-linked immunosorbent assay for the quantitative determination of T3 levels 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", and can readily enter target tissue. The basic thyroid function panel is composed of TSH, TT3 (free + protein-bound), TT4 (free + protein-bound), free T3 (fT3), and free T4 (fT4) detection tests[3,7,8].

T3 tests can be used to aid in the detection and diagnosis of thyroid disease including hyperthyroidism, thyrotoxicosis, and Graves' disease, and to monitor both 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 to give the most accurate measurement of thyroid status possible[7].

TEST PRINCIPLE

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

The *RecombiLISA* Total Triiodothyronine (T3) is comprised of three key components:
1) Solid microwells pre-coated with anti-T3 antibody,
2) T3 Standards,
3) Liquid conjugate comprised of horseradish peroxidase conjugated to T3 (T3 Enzyme Conjugate Reagent).

During the assay, the test specimen and the T3 Enzyme Conjugate Reagent are simultaneously incubated with the microwells coated with anti-T3 antibody. The T3 in the patient specimen will then compete with the T3 in the T3 Enzyme Conjugate Reagent for binding to the anti-T3 antibody coated on the microwell surface.

Unbound material is then removed by washing. The presence of the T3-HRP bound to the microwell surface is shown by the development of a blue color upon addition of the Substrate. The reaction is then terminated with Stop Solution and the absorbance 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 patient specimen, within the dynamic range of the assay.

A standard curve is generated on graph paper by plotting the absorbance value for each T3 standard measured at a wavelength of 450/620-690 nm on one axis, versus the concentration of the T3 standard on the other, and drawing a best-fit line. The T3 concentration in each specimen is then determined directly using this curve.

MATERIALS AND REAGENTS

Materials and reagents required but not provided in the kit

1. Micropipettes capable of dispensing appropriate volumes
2. Microplate reader with a bandwidth of 10 nm of less and an optical density range of 0-3 OD or greater at 450 nm wavelength is acceptable
3. Vortex mixer or equivalent
4. Absorbent paper for blotting the microwells
5. Graph paper
6. Timer
7. Distilled or de-ionized water
8. Disposable reagent troughs
9. Incubator

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Anti-T3 Ab Coated Microwells	8 wells x 12 strips	E1010W
2	T3 Standards: S1 (0 ng/mL)	1 mL	E1010S1
3	S2 (0.5 ng/mL)	1 mL	E1010S2
4	S3 (1.0 ng/mL)	1 mL	E1010S3
5	S4 (2.5 ng/mL)	1 mL	E1010S4
6	S5 (5.0 ng/mL)	1 mL	E1010S5
7	S6 (10.0 ng/mL)	1 mL	E1010S6
8	HRP-T3 Conjugate Concentrate (20X)	1.2 mL	E1010H
9	Enzyme Conjugate Diluent	2 vials x 7.5 mL	E1010ED
10	Wash Buffer Concentrate (40X)	25 mL	WE4000-25
11	Substrate	12 mL	TME2000
12	Stop Solution	7.5 mL	SE1000
13	Product Insert	1	PI-E1010

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. All the reagents are stable through the expiration date printed on the label if not opened. Ensure that the reagents are brought to room temperature before opening. Reseal the microwells after removing the desired number of wells. Place unused wells in the resealable plastic bag provided and return to 2-8°C.

SPECIMEN COLLECTION AND PREPARATION

- Serum specimens should be prepared from whole blood obtained by acceptable venipuncture technique.
- Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
- If a specimen is not tested immediately, refrigerate at 2-8°C. If a storage period greater than three days is anticipated, the specimen should be frozen (-20°C). Avoid repeated freeze-thawing of specimens. 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 prior to performing the assay.
- Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS

1. Bring all reagents to room temperature (18-28°C).
2. Prepare Enzyme Conjugate Reagent: Add 0.1 mL of Enzyme Conjugate Concentrate to 2.0 mL of Enzyme Conjugate Diluent (1:20 dilution), and mix well. The amount of conjugate diluted is dependent on your assay size. The Conjugate Reagent is stable at 4°C for 7 days.
3. Prepare Wash Buffer: add 25 mL of Wash Buffer Concentrate to 1000 mL of distilled water and mix well. The wash solution is stable at room temperature (18-28°C) for two months.
4. Determine the number of microwells needed and mark a data sheet with appropriate information. Standards and Controls require at least one well each; however, it is recommended that standards and controls be run in duplicate to ensure accuracy. A blank well is not required.
5. Adjust the incubator to 37°C.

ASSAY PROCEDURE

1. Remove the desired number of microwells and secure them in the microplate frame. Reseal un-used strips.
2. Add 50 µL of T3 standards and patient specimens into the assigned microwells.
3. Add 100 µL of T3 Enzyme Conjugate Reagent into each well.
4. Shake on a vortex mixer for 30 seconds to completely mix the liquid within the wells and cover the plate with a lid.
5. Incubate the microplate at 37°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 diluted wash buffer and rock gently for 20-30 seconds. Discard the wash solution completely. Repeat 4more 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 working wash buffer and soak for 20-30 seconds. Aspirate all wells completely. Repeat 4 more times.
7. Add 100 µL of Substrate into each well. Gently shake the microplate for 20 seconds to ensure thorough mixing.
8. Incubate the microwells at room temperature (18-28°C) in the dark for 20 minutes.
9. Stop the reaction by adding 50 µL of Stop Solution into each well. Gently mix for 15 seconds. Pipette 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 and measure the absorbance (OD) of each well within 15 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 T3 Standards or specimens	50 µL
3.	Add T3 Enzyme Conjugate Reagent	100 µL
4. 5.	Shake Incubate	30 seconds 37°C, 60 minutes
6.	Wash: manual or automatic	5 times
7.	Add Substrate.	100 µL
8.	Incubate in dark	18-28°C, 20 minutes
9.	Add Stop Solution. Gently mix	50 µL 15 seconds
10.	Read the result	450/620-690 nm within 15 minutes

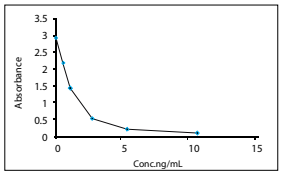
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A450/620-690) for each set of standards and patient specimens when duplicates are used.
2. Construct a curve by plotting the mean value (or subtracted mean value) obtained for each standard against its concentration on graph paper with absorbance values on the vertical Y axis, and concentrations on the horizontal X axis. Draw a best fit line through the points.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of T3 in ng/mL from the curve.
   Alternatively, if software is used, calculate the concentration of T3 following the software menu.
4. 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 run are shown below:

Cal ID	Conc. (ng/mL)	OD
S1	0.0	2.958
S2	0.5	2.244
S3	1.0	1.449
S4	2.5	0.568
S5	5.0	0.208
S6	10.0	0.118



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

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 T3 can be used as initial guideline ranges only:

Sample Number	619
Average Value (ng/mL)	1.45
Standard Deviation	0.33
Normal Range	0.8-2.1

PEFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

OD values from 10 replicates of the '0' standard were run in a single assay on three lots of the *RecombiLISA* Total Triiodothyronine (T3) ELISA, and the mean and standard deviation (SD) were calculated. The analytical sensitivity of the test was interpolated from the dose response curve (DRC). The analytical sensitivity of the *RecombiLISA* Total Triiodothyronine (T3) ELISA was determined to be 0.2 ng/mL at 2SD.

2. Specificity

Cross-reactivity with the *RecombiLISA* Total Triiodothyronine (T3) ELISA was evaluated by spiking T3 and rT3 into the '0" standard. The results are listed in the following table:

Compound	Concentration (ng/mL)	Measured Value (ng/mL)	Cross-Reactivity (%)
T4	500	1.44	< 0.29
rT3	500	0.24	< 0.05

3. Accuracy

The accuracy of the *RecombiLISA* Total Triiodothyronine (T3) ELISA was determined by comparison with a commercial ELISA as a reference. A total number of 210 patient specimens with a range of 0.4-8.5-25 ng/mL were tested with both kits. The results are listed in the table below:

Number of Specimens	Least Square Regression	Corr. Coef. (r)
210	y = 1.018x + 0.155	0.963

4. Precision

a. **Intra-assay precision:** Twenty replicates of each of two pools of human sera (low and high concentrations) were tested in the same assay. The mean, SD, and coefficient of variation (CV) were determined. The results are showed in the following table:

Sample	N	Mean (ng/mL)	SD	CV
Low	20	1.76	0.04	2.25%
High	20	3.96	0.12	3.10%

b. **Inter-Assay Precision:** Two pools of human sera (low and high concentrations) were tested in 20 separate runs. The mean, SD, and CV were determined. The results are shown in the following table:

Sample	N	Mean (ng/mL)	SD	CV
Low	20	1.05	0.09	8.57%
High	20	1.95	0.07	3.58%

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to check assay performance. Control containing sodium azide cannot be used. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance. This kit does not include serum controls.

WARNING AND PRECAUTIONS

For *in Vitro* Diagnostic

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired kits.
3. Bring all reagents to room temperature (18-28°C) before use.
4. Do not use the components of any other type of 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. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
10. At the beginning of each incubation and after adding Stopping Solution, gently rock the microwells to ensure thorough mixing. Avoid the formation of air bubbles which results in inaccurate absorbance values. Avoid splashing liquid while rocking or shaking the wells.
11. Do not allow the microwells to dry between the end of the washing operation and the reagent distribution.
12. The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate.
13. The substrate must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The Substrate must be stored in the dark.
14. Use a new dispensing tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
15. The wash procedure is critical. Wells must be aspirated completely before adding the Wash Buffer or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance values.
16. 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 the level of T3 in serum from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The test is limited to the detection of T3 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 malfunction may give rise to abnormal T3 concentrations in serum. Significant changes of concentrations of T3 and T4 have been reported in patients with 'non-thyroidal illness' (NTI).
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. Some patient specimens containing auto-antibodies to thyroid hormones may interfere with T3 test results.

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Index of Symbols

	See instructions for use		Use by
REF	Catalog #	STD	Standard
LOT	Lot number	CONJ	Conjugates
N	Tests per kit	SUBS	Substrate
	Do not reuse	STOP SOLN	Stop solution
	Manufacturer	WASH BUFF	Wash buffer
	Date of manufacture	MICROWELLS	Coated microwells
2°C 8°C	Store between 2-8°C	EC REP	Authorized representative

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