



Instructions for Use

Triiodothyronine (T3) ELISA

IVD



REF EIA-1780

 96



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**Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.**

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For In Vitro Diagnostic Use

Store at 2 °C to 8 °C.

1 INTENDED USE

This Triiodothyronine (T3) ELISA is intended for the quantitative determination of triiodothyronine (T3) concentration in human serum. This test is useful in the diagnosis and treatment of thyroid diseases such as hyperthyroidism.

2 INTRODUCTION

The thyroid hormones, thyroxine (T4) and 3, 5, 3' triiodothyronine (T3) circulate in the bloodstream mostly bound to the plasma protein, thyroxine binding globulin (TBG).¹ The concentration of T3 is much less than that of T4, but its metabolic potency is much greater.^{2,3} These as well as other hormones are produced by the thyroid gland, situated at the level of the thyroid cartilage on either side of the larynx. The thyroid gland and associated hormones are a major component of the endocrine system. They exert powerful and essential regulatory influences on growth, differentiation, cellular metabolism, and general hormonal balance of the body, as well as on the maintenance of metabolic activity and the development of the skeletal and organ systems.

T3, T4 and TSH determination are important factors in thyroid disease diagnosis. T3 determination is useful in monitoring both patients under treatment for hyperthyroidism, and patients who have discontinued anti-thyroid drug therapy. It is especially valuable in distinguishing between euthyroid and hyperthyroid subjects.⁴

T3 measurement has uncovered a variant of hyperthyroidism in thyrotoxic patients with elevated T3 values and normal T4 values⁵. Likewise, an increase in T3 without an increase in T4 is frequently a forerunner of recurrent thyrotoxicosis in previously treated patients. In addition to hyperthyroidism, T3 levels are elevated in women who are pregnant, and in women receiving oral contraceptives or estrogen treatment.

The clinical significance of T3 is also evident in patients where euthyroidism is attributable only to normal T3, although their T4 values are subnormal.⁶

3 PRINCIPLE OF THE ASSAY

In this T3 EIA, a second antibody (goat anti-mouse IgG) is coated on a microtiter wells. A measured amount of patient serum, a certain amount of mouse monoclonal Anti-T3 antibody, and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the mouse anti-T3 antibody is bound to the second antibody on the wells. T3 and the enzyme conjugated-T3 compete for the limited binding sites on the anti-T3 antibody. After a 60 minute incubation at room temperature, the wells are washed 5 times by water to remove unbound T3 conjugate. A solution of TMB is then added and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present, and is inversely related to the amount of unlabeled T3 standards assayed in the same way. The concentration of T3 in the unknown sample is then calculated.⁷⁻¹¹

4 REAGENTS AND MATERIALS PROVIDED

1. **Antibody-Coated Wells** (1 plate, 96 wells)
Microtiter wells coated with goat anti-mouse IgG.
2. **Enzyme Conjugate Concentrate** (11X, 1.3 mL)
Contains T3-HRPO Conjugate, with TRIS buffer, pH = 7.60 and ProClin-300.
3. **Enzyme Conjugate Diluent** (1 bottle, 13 mL)
Contains ANS, TRIS buffer, pH=7.60 and ProClin-300.
4. Reference **Standard Set** (1 mL/vial)
Contains 0 - 0.75 - 1.5 - 3.0 - 6.0 and 10.0 ng/mL triiodothyronine in T3/T4-free stripped human serum and ProClin-300.
5. **T3 Antibody Reagent** (1 bottle, 7 mL)
Contains mouse monoclonal anti-T3 in phosphate buffer, pH = 7.60 and ProClin-300.
6. **TMB Reagent** (1 bottle, 11 mL)
Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
7. **Stop Solution** (1N HCl) (1 bottle, 11 mL)
Contains diluted hydrochloric acid.

5 MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes: 25 µL, 100 µL, 200 µL, and 1 mL
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450 nm.
5. Absorbent paper
6. Graph paper
7. Vortex mixer or equivalent
8. Quality control material (e.g. BioRad Lyphochek Control sera)

6 WARNINGS AND PRECAUTIONS

1. **CAUTION:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.¹²
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
6. Do not pipette reagents by mouth.
7. For in vitro diagnostic use.

7 STORAGE CONDITIONS

1. Store the unopened kit at 2 °C to 8 °C upon receipt and when not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

8 SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture technique and the serum should be separated from the red cells as soon as practical. Avoid grossly hemolytic, lipemic, or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with the test procedure.
3. Specimens should be capped and may be stored for up to 48 hours at 2 °C to 8 °C prior to assaying. Specimens held for a longer time (up to 6 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

9 REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18 °C to 25 °C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. To prepare **Working T3-HRPO Conjugate Reagent:** add 0.1 mL of T3-HRPO Conjugate Concentrate (11X) to 1.0 mL of T3 Conjugate Diluent (1:10 dilution), and mix well.
The amount of conjugated diluted depends on the assay size.
The Working Conjugate Reagent is stable at 4 °C for at least 24 hours.

10 PROCEDURAL NOTES

1. **Manual Pipetting:** It is recommended that no more than 32 wells be used for each assay run. A multi-channel pipette is recommended.
2. **Automated Pipetting:** A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

11 INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

12 ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Pipette 50 μ L of standards, specimens, and controls into appropriate wells.
3. Dispense 50 μ L of T3 Antibody Reagent into each well. Mix thoroughly for 30 seconds.
4. Add 100 μ L of **Working Conjugate Reagent** into each well. Mix thoroughly for 30 seconds.
It is important to have complete mixing in step 3 and 4.
5. Incubate at room temperature (18 °C to 25 °C) for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 μ L of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature, in the dark, for 20 minutes.
11. Stop the reaction by adding 100 μ L of Stop Solution to each well.
12. Gently mix for 30 seconds. ***Ensure that all of the blue color changes completely to yellow.***
13. Read absorbance at 450nm with a microtiter plate reader **within 15 minutes**.

13 CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD 450) from the duplicate set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of T3 in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

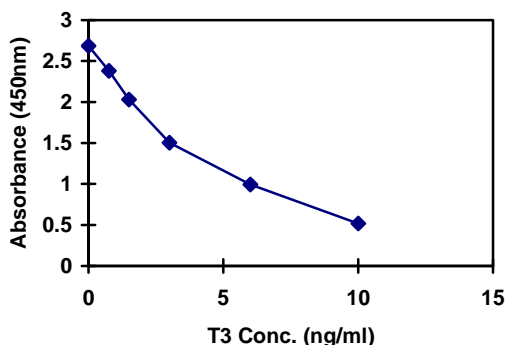
14 CALIBRATION OF ASSAY

The T3 standards are calibrated against the Diagnostic Products Corporation's Total T3 Coat-A-Count RIA test. The accuracy of this calibration is $100 \pm 5\%$. Therefore, the accuracy of patient samples assayed with the Triiodothyronine (T3) ELISA (EIA-1780) can vary by $\pm 5\%$.

14.1 Example of Standard Curve

Results of a typical standard run with optical density readings at 450 nm shown on the Y-axis against Total T3 concentrations (ng/mL) shown on the X-axis, are presented below. **NOTE:** the standard curve is for illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve for each assay run. Additionally, the absorbance (450 nm) values can be varied due to incubation at different room temperature in different laboratories.

Total T3 (ng/mL)	Absorbance (450nm)
0.0	2.685
0.75	2.381
1.5	2.028
3.0	1.502
6.0	0.992
10.0	0.518



15 EXPECTED VALUES

The Triiodothyronine (T3) ELISA (EIA-1780) was utilized in a study of 41 hypothyroid, 64 euthyroid, and 49 hyperthyroid patients (as determined by hospital laboratory analysis) in one geographic location. The range, as determined from lowest to highest patient value of samples tested, was 0.14 ng/mL to 6.20 ng/mL and yielded the following ranges:

Hypothyroid: < 0.8 ng/mL
 Euthyroid: 0.8 – 1.9 ng/mL
 Hyperthyroid: > 1.9 ng/mL

These ranges correspond to those suggested by other commercial manufacturers. In general, total serum T3 levels will tend to parallel the variations in serum levels of the major binding protein, thyroxine binding globulin (TBG).

Elevated T3 levels may be encountered in hypothyroid individuals receiving replacement therapy.^{13,14} Inadequate iodine uptake may also cause elevated serum levels of T3.¹⁵ T3 levels are lower than normal in cord blood and also tend to be lower in the elderly.^{16,17.}

It is recommended that laboratories adjust values to reflect geographic and population differences specific to the patients they are testing.

The following conditions and treatments may alter TBG levels¹⁸:

Increased serum TBG

- Pregnancy
- Estrogen therapy (including oral contraceptives)
- Phenothiazines
- Viral hepatitis
- Acute intermittent porphyria
- Myxoedema
- Hereditary causes

Decreased Serum TBG

- Androgens
- Cortisosteroids
- Anabolic steroids
- Active acromegaly
- Nephritic syndromes
- Stress, major illness, or surgery
- Malnutrition

16 PERFORMANCE CHARACTERISTICS

16.1 Accuracy

A statistical study comparing the T3 assay (EIA-1780) with the Abbott AxSym Total T3 kit utilized 67 patient samples (range = 0.32 – 5.9 ng/mL), and demonstrated good correlation with the kit as shown below:

N = 67

Correlation coefficient = 0.906

Slope = 0.920

Intercept = -0.229

(EIA-1780) Mean = 1.24 ng/mL

Abbott Mean = 0.91 ng/mL

An additional study comparing the T3 kit (EIA-1780) with the Monobind Total T3 assay (n = 80, range = 0.14 – 6.2 ng/mL) also correlated well as shown below:

N = 80

Correlation coefficient = 0.993

Slope = 1.004

Intercept = -0.084

(EIA-1780) Mean = 1.21 ng/mL

Monobind Mean = 1.13 ng/mL

16.2 Sensitivity

The sensitivity of this Triiodothyronine (T3) ELISA test is defined as the lowest concentration of T3 that can be distinguished from 0 ng/mL as calculated from the 95% confidence limits of the 0 ng/mL standard absorbance. This method will reliably detect T3 concentrations as low as 0.2 ng/mL.

16.3 Precision

16.3.1 Intra-Assay Precision

Within-run precision was determined by replicate determinations of three different control sera in 1 assay.

Serum Sample	1	2	3
Number of Replicates	20	20	20
Mean T3 (ng/mL)	0.85	2.48	4.41
Standard Deviation	0.08	0.10	0.14
Coefficient of Variation (%)	9.6%	4.1%	3.2%

16.3.2 Inter-Assay Precision

Between-run precision was determined by replicate measurements of three different serum samples over a series of individually calibrated assays.

Serum Sample	1	2	3
Number of Replicates	20	20	20
Mean T3 (ng/mL)	0.79	2.53	4.10
Standard Deviation	0.08	0.08	0.06
Coefficient of Variation (%)	10.3%	3.2%	1.4%

16.4 Recovery and Linearity Studies

16.4.1 Recovery

Various patient serum samples of known T3 levels were combined and assayed in duplicate. The mean recovery was 106%.

Expected Concentration (ng/mL)	Observed Concentration (ng/mL)	% Recovery
5.05	4.1	99%
2.71	2.73	101%
1.46	1.52	104%
0.68	0.65	96%
Mean Recovery #1 = 100 %		
5.81	6.16	106%
3.02	3.17	105%
1.54	1.75	114%
0.71	0.84	119%
Mean Recovery #2 = 111 %		

16.4.2 Linearity

Two samples were serially diluted with zero T3 standard to determine linearity. The mean recovery was 99%.

#	Dilution	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Expected
1.	Undiluted	----	6.06	----
	1:2	3.03	3.05	101%
	1:4	1.51	1.46	97%
	1:8	0.75	0.68	91%
Average = 96%				
2.	Undiluted	----	5.66	----
	1:2	2.83	2.86	101%
	1:4	1.42	1.55	109%
	1:8	0.71	0.67	94%
Average = 101%				

16.5 Specificity

The following hormones were tested for cross-reactivity:

HORMONE TESTED	CONCENTRATION	PRODUCED COLOR INTENSITY EQUIVALENT TO T3 (ng/mL)
<i>Triiodo-L-Thyronine</i>	1.0 ng/mL	1.0
	3.0 ng/mL	3.0
	6.0 ng/mL	6.0
	8.0 ng/mL	8.0
<i>Triiodo-D-Thronine</i>	0.5 ng/mL	0.45
	1.0 ng/mL	0.81
	2.0 ng/mL	2.37
	4.0 ng/mL	4.14
	8.0 ng/mL	7.97

HORMONE TESTED	CONCENTRATION	PRODUCED COLOR INTENSITY EQUIVALENT TO T3 (ng/mL)
<i>L-Thyroxine (T4)</i>	4.5 µg/dL	0
	9.0 µg/dL	0.22
	18.0 µg/dL	0.53
	36.0 µg/dL	1.35
<i>D-Thyroxine (T4)</i>	2.0 µg/dL	0.32
	4.0 µg/dL	0.40
	8.0µg/dL	0.55
<i>Triiodothyroacetic Acid</i>	2.5 ng/mL	3.47
	5.5 ng/mL	6.53
	10.0 ng/mL	>8.0
	20.0 ng/mL	>8.0
	100.0 ng/mL	>8.0
<i>Monoiodotyrosine</i>	1,000 ng/mL	0
	10,000 ng/mL	0.17
	50,000 ng/mL	1.96
<i>Diiodotyrosine</i>	1,000 ng/mL	0.08
	10,000 ng/mL	0.12
	50,000 ng/mL	0.58
<i>Methimazole</i>	1,000 ng/mL	0.05
	50,000 ng/mL	0.06
	500,000 ng/mL	0.25
<i>5,5'-Diphenylhydantoin</i>	1,000 ng/mL	0
	10,000 ng/mL	0.03
<i>Phenylbutazone</i>	10,000 ng/mL	0
	50,000 ng/mL	0
	1,000,000ng/mL	0.36
<i>6-n-Propyl-2-Thiouracil</i>	10,000 ng/mL	0.10
	100,000 ng/mL	1.48
	250,000 ng/mL	1.66
<i>Salicylic Acid</i>	5,000 ng/mL	0
	500,000 ng/mL	0
	1,000,000 ng/mL	0.28
<i>Acetylsalicylic Acid</i>	5,000 ng/mL	0
	500,000 ng/mL	0
	1,000,000 ng/mL	0

17 LIMITATIONS OF THE PROCEDURE

1. **Important Note:** Individuals receiving thyroid replacement therapy, such as triiodothyroacetic acid or triiodothyropionic acid, may give falsely high T3 values in this test. Numerous other conditions unrelated to thyroid disease may cause abnormal T3 values (see Expected Results).
2. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
3. Serum samples with T3 concentrations greater than 10 ng/mL should be diluted with the Zero Standard to fit into the assay range, and re-assayed. The obtained value should then be multiplied by the dilution factor to obtain the true serum value.
4. Icteric samples with bilirubin values as high as 5 mg/dl do not affect the assay.
5. Added hemoglobin levels of up to 100 mg/dL showed no effect on the T3 value.
6. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
7. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

18 QUALITY CONTROL

Good laboratory practice requires that low, medium and high quality control specimens (controls) be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

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SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
	<i>In vitro</i> diagnostic medical device *	<i>In-vitro</i> -Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
	Catalogue number *	Artikelnummer *	Numero di Catalogo	Número de catálogo	Référence de catalogue
	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservación	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
<i>Distributed by</i>	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
<i>Content</i>	Content	Inhalt	Contenuto	Contenido	Contenu
<i>Volume/No.</i>	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité