Instructions for Use

Total Thyroxine (T4) ELISA

IVD

CE

REF EIA-1781

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(en 030104 CE)

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

For the quantitative determination of total thyroxine (T4) concentration in human serum. The test is useful in the diagnosis and treatment of thyroid disorders.

2 EXPLANATION OF THE TEST

The thyroid hormones thyroxine (T4) and triiodothyronine (T3), are synthesized and stored in the thyroid gland and circulate in the bloodstream mostly bound to the plasma protein, thyroxine binding globulin (TBG).[1] 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.

Proteolytic cleavage of follicular thyroglobulin releases T4 into the bloodstream. Greater than 99% of T4 is reversibly bound to three plasma proteins in blood - thyroxine binding globulin (TBG) binds 70%, thyroxine binding pre-albumin (TBPA) binds 20%, and albumin binds 10%.[2,3] Approximately 0.03% of T4 is in the free, unbound state in blood at any one time.

Diseases affecting thyroid function may present a wide array of confusing symptoms[1]. Measurement of total T4, TSH, Free T3 and Free T4 by immunoassay are reliable and convenient methods to determine the presence of thyroid disorders in patients.[4,5] Increased levels of T4 have been found in hyperthyroidism due to Grave's disease and Plummer's disease and in acute and subacute thyroiditis. Low levels of T4 have been associated with congenital hypothyroidism, myxedema, chronic thryoiditis (Hashimoto's disease), and with some genetic abnormalities.

3 PRINCIPLE OF THE ASSAY

To measure T4 by competitive immunoassay techniques, a sample of serum or plasma containing the T4 to be quantified is mixed with labeled T4 and T4 antibody.[6,7] The labeled T4 contains 8-anilino-1-napthalene sulfonic acid (ANS) to inhibit binding of T4 to serum proteins, which would otherwise interfere with the assay. During incubation, a fixed amount of labeled T4 competes with the unlabeled T4 in the sample, standard, or quality control serum for a fixed number of binding sites on the specific T4 antibody.

Separation of the unbound T4 from antibody-bound T4 and the subsequent measurement of the labeled fraction of the bound phase completes the test. By comparing results of the unknown sample with those obtained from a series of T4 calibrators, an accurate measurement of the T4 concentration in the sample can be obtained.

In the T4 ELISA (EIA-1781), antibody to T4 is coated on a solid phase (microtiter well). A measured amount of patient serum and a constant amount of T4 labeled with horseradish peroxidase are added. During incubation, T4 in the patient sample and enzyme-labeled T4 compete for the limited binding sites on the T4 antibody. After a 60-minute incubation at room temperature, the solid phase is washed with water to remove unbound-labeled T4. A solution of tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the resulting yellow color 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 T4 in the patient sample. By reference to a series of calibrators processed in the same way, the concentration of T4 in the unknown sample is determined.

4 REAGENTS AND MATERIALS PROVIDED

1. **Antibody-Coated Wells** (1 plate, 96 wells)
   Microtiter wells coated with sheep anti-T4.
2. **Enzyme Conjugate Concentrate** 11X (1.3 mL)
   Contains T4-HRP Conjugate
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, 2.0, 5.0, 10.0, 15.0 and 25.0 µg/dL in T3/T4-free stripped human serum, 1 set, liquid, ready-to-use.
5. **TMB Reagent** (1 bottle, 11 mL)
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
6. **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 and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[21]
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. 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.
10. For in vitro diagnostic use.

7 STORAGE CONDITIONS

1. Store the unopened kit at 2 °C - 8 °C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2 °C - 8 °C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

8 INSTRUMENTATION

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

9 SPECIMEN COLLECTION AND PREPARATION

1. Serum is the sample of choice. 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 – 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.

10 REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18 °C – 25 °C) before use, and should be mixed by gentle inversion or swirling. Do not induce foaming.
2. To prepare **Working T4-HRPO Conjugate Reagent:**
   add 0.1 mL of T4-HRPO Conjugate Concentrate (11x) to 1.0 mL of T4 Conjugate Diluent (1:10 dilution), and mix well. The amount of conjugate diluted depends on the assay size.
   The Working Conjugate Reagent is stable at 4 °C for 24 hours.

11 PROCEDURAL NOTES

1. Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes. 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.

12 ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Pipette **25 µL** of *standards, specimens, and controls* into appropriate wells.
3. Add 1**00 µL** of *Working Conjugate Reagent* into each well.
4. Mix thoroughly for 30 seconds.
5. Incubate at room temperature (18 °C – 25 °C) for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled H_2O.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense **100 µL** 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 µL** 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 450 nm with a microtiter plate reader **within 15 minutes**.

13 CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD 450 nm) 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 log-log 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 T4 in µg/dL 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.

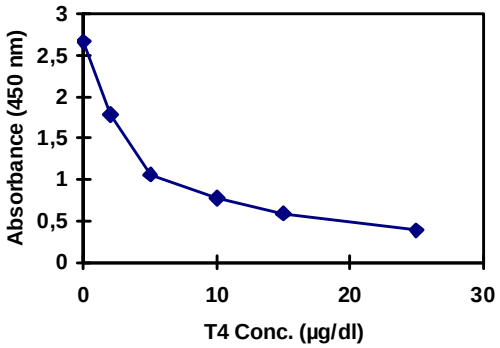
13.1 Calibration of Assay

The T4 standards are calibrated against the Diagnostic Products Corporation's Total T4 Coat-A-Count RIA test. The accuracy of this calibration is 100 ± 5%. Therefore, the accuracy of patient samples assayed with the Total T4 ELISA (EIA-1781) can vary by ± 5%.

13.2 Example of Standard Curve

Results of a typical standard run with optical density readings at 450 nm shown on the Y-axis against Total T4 concentrations (µg/dL) 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 T4 (µg/dL)	Absorbance (450 nm)
0	2.667
2	1.786
5	1.060
10	0.778
15	0.591
25	0.384



14 EXPECTED VALUES

The T4 ELISA (EIA-1781) was utilized in a study of 200 euthyroid patient samples (as determined by hospital laboratory analysis) in one geographic location and yielded a normal range of 5.0 to 13.0 µg/dL.

The range was determined by the observed values and corresponds to those suggested by other commercial manufacturers.

It is recommended that laboratories adjust values to reflect geographic and population differences.

15 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.

16 PERFORMANCE CHARACTERISTICS

16.1 Accuracy

A statistical study using 82 patient samples (range = 1.3 – 24.5 µg/dL) demonstrated good correlation of results with Abbott's AxSym® Total T4 Kit:

N = 82
Correlation Coefficient = 0.954
Slope = 0.914
Intercept = 1.049
EIA-1781 Mean = 9.9 µg/dL
Abbott AxSym Mean = 10.1 µg/dL

Another study using 107 patient samples (range = 2.2 – 29.7 µg/dL) demonstrated good correlation of results with Monobind's Total T4 EIA kit as shown below:"

N = 107
Correlation Coefficient = 0.978
Slope = 0.938
Intercept = O.485
BioCheck Mean = 10.1 µg/dl
Monobind Mean = 10.0 µg/dl

16.2 Sensitivity

The minimum detectable concentration of T4 that can be defined by this assay is 0.5 µg/dL.

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	26	26	26
Mean T4 (µg/dL)	3.95	8.65	20.51
Standard Deviation	0.17	0.27	0.80
Coefficient of Variation (%)	4.3%	3.1%	3.9%

16.3.2 Inter-Assay Precision

Between-run precision was determined by replicate measurements of three different serum samples in several different assays.

Serum Sample	1	2	3
Number of Replicates	26	26	26
Mean T4 (µg/dL)	4.29	8.71	19.01
Standard Deviation	0.19	0.35	0.45
Coefficient of Variation (%)	4.5%	4.0%	2.4%

16.4 Recovery and Linearity Studies

16.4.1 Recovery

Various patient serum samples of known T4 levels were combined and assayed in duplicate. The mean recovery was 96.7%.

Expected Concentration (µg/dL)	Observed Concentration (µg/dL)	% Recovery
19.49	20.06	102.9%
8.36	6.98	83.5%
6.17	6.20	100.5%
4.11	3.58	87.1%
3.36	3.67	109.2%
1.66	1.52	91.6%
	Mean Recovery #1 = 95.8 %	
21.52	22.04	102.4%
14.85	13.78	92.8%
6.70	6.89	102.8%
1.70	1.56	91.8%
	Mean Recovery #2 = 97.5%	

16.4.2 Linearity

Two samples were serially diluted with T3/T4-free human serum to determine linearity. The mean recovery was 98.8%.

#	Dilution	Expected Conc. (µg/dL)	Observed Conc. (µg/dL)	% Expected
1.	Undiluted	----	15.12	----
	1:2	7.56	7.50	99.2%
	1:4	3.78	3.80	100.5%
	1:8	1.89	1.74	91.9%
			Average = 97.2%	
2.	Undiluted	----	16.74	----
	1:2	8.37	8.86	105.7%
	1:4	4.19	4.18	99.8%
	1:8	2.09	1.80	86.0%
			Average = 100.4%	

16.5 Specificity

The following substances were tested for cross-reactivity:

Material TESTED	CONCENTRATION	COLOR INTENSITY EQUIVALENT to T4 (µg/dL)
L-Thyroxine (T4)	2 µg/dL 4 µg/dL 6 µg/dL 8 µg/dL	2.22 3.80 5.53 8.79
D-Thyroxine (T4)	2 µg/dL 4 µg/dL 6 µg/dL 8 µg/dL	2.61 4.38 6.84 9.13
Triiodo-L-Thyronine	0.5 µg/dL 2 µg/dL 4 µg/dL 6 µg/dL 8 µg/dL	0.20 0.47 0.68 1.36 1.58
Triiodo-D-Thronine	0.5 µg/dL 2 µg/dL 4 µg/dL 6 µg/dL 8 µg/dL	0.16 0.40 0.60 1.20 1.40
Triiodothyroacetic Acid	0.5 µg/dL 2 µg/dL 6 µg/dL 10 µg/dL 25 µg/dL 100 µg/dL	0.00 0.05 0.10 0.25 0.60 2.90
Monoiodotyrosine	1,000 µg/dL 5,000 µg/dL	0.00 0.27
Diiodotyrosine	1,000 µg/dL 5,000 µg/dL	0.00 0.00
Methimazole	5,000 µg/dL 50,000 µg/dL 2,000,000 µg/dL	0.00 0.00 0.00
5,5'-Diphenylhydantoin	1,000 µg/dL 50,000 µg/dL 250,000 µg/dL	0.00 0.00 0.00
Phenylbutazone	5,000 µg/dL 1,000,000 µg/dL	0.00 0.00
6-n-Propyl-2-Thiouracil	1,000 µg/dL 10,000 µg/dL 25,000 µg/dL	0.00 0.00 0.00
Salicylic Acid	500 µg/dL 50,000 µg/dL 100,000 µg/dL	0.00 0.00 0.00
Acetylsalicylic Acid	5,000 µg/dL 50,000 µg/dL	0.32 0.42

17 LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with good laboratory practice and adherence to the package insert instructions.
2. 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.
3. Serum samples with T4 concentrations greater than 25 µg/dL should be reported as such. If further quantitation is desired, the sample should be diluted with the Zero Standard 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. Additionally, added hemoglobin levels of up to 100 mg/dl showed no effect on the T4 value.
5. Total serum T4 values may be influenced by a variety of factors other than thyroid malfunction. High TSH levels, pregnancy, estrogen therapy, oral contraceptives, heparin, phenytoin and propanolol may all produce invalid results.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

18 REFERENCES / LITERATURE

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1 IM KIT ENTHALTENE MATERIALIEN

1. **Antibody-Coated Wells**
   Schaf-anti-T4 beschichtete Microtiter Platte, 96 Wells
2. **Enzyme Conjugate Concentrate**
   Enzymkonjugat, Konzentrat (11 x) ; 1,3 mL.
3. **Enzyme Conjugate Diluent**
   Enzymkonjugat-Verdünnungslösung, 13 mL
4. Reference **Standard** Set
   T4 Standards: 0, 2, 5, 10, 15, und 25 µg/dL, 1 Set, je 1.0 mL, gebrauchsfertig.
5. **TMB Reagent**
   TMB Substrat, 11 mL
6. **Stop Solution**
   Stopplösung (1N HCl), 11 mL

2 BENÖTIGTE MATERIALIEN DIE NICHT IM KIT ENTHALTEN SIND

1. Destilliertes Wasser
2. Präzisions Pipette: 25 µL, 100 µL, 200 µL und 1.0 mL
3. Einmal-Pipettenspitzen
4. Mikrotiter Platten Lesegerät (450 nm Wellenlänge)
5. Saugpapier oder Papierhandtuch
6. Lineares Millimeterpapier
7. Vortex Mixer

3 AUFBEWAHRUNG DER REAGENZIEN

Ungeöffnete Kits sollten bei 2 °C - 8 °C gelagert werden. Das Verfallsdatum befindet sich auf dem Kitetikett.

4 PROBENENTNAHME

Für diesen Test sollten nur Serumproben ohne Zusätze verwendet werden.

Blut durch Venenpunktion entnehmen, gerinnen lassen und das Serum durch Zentrifugation bei Raumtemperatur abtrennen.

5 VORBEREITUNG DER REAGENZIEN

1. Alle Reagenzien vor Gebrauch auf Raumtemperatur (18 °C - 25 °C) bringen.
2. **Herstellung der gebrauchsfertigen Konjugat-Lösung:**
   Verdünnung 1+10:
   100 µL T4 Konjugat-Konzentrat (11x) mit 1 mL T4 Konjugat-Verdünnungslösung auffüllen.
   Gut mischen.
   Bitte beachten:
   Stellen Sie nur die für den Test benötigte Menge an gebrauchsfertigem Konjugat her.
   Das gebrauchsfertige Konjugat sollte innerhalb von 24 Stunden verbraucht werden.

6 TESTDURCHFÜHRUNG

1. Die benötigte Anzahl an Wells in der Halterung befestigen.
2. **25 µL** *Standards, Kontrollen und Proben* in die entsprechenden Vertiefungen pipettieren.
3. **100 µL** *gebrauchsfertiges Enzymkonjugat* in alle Vertiefungen geben.
4. Ca. 30 Sek. gründlich mischen. Eine vollständige Vermischung der Reagenzien ist sehr wichtig!
5. 60 Minuten bei Raumtemperatur (18 °C - 25 °C) inkubieren.
6. Inkubationslösung zügig abschütten.
7. Vertiefungen 5-mal mit destilliertem Wasser waschen.
8. Nach dem letzten Waschen Wassertropfen aus den Vertiefungen durch Ausklopfen auf saugfähigem Papier entfernen. (Bitte kein Leitungswasser verwenden!)
9. **100 µL** *TMB Substrat* in alle Vertiefungen geben. 5 Sekunden vorsichtig mischen.
10. 20 Minuten bei Raumtemperatur im Dunkeln inkubieren.
11. Die Reaktion durch Zugabe von **100 µL** *Stop Solution* stoppen.
12. 30 Sekunden vorsichtig mischen. **Es ist wichtig, dass der Farbumschlag von Blau zu Gelb vollständig erfolgt.**
13. Die Extinktion bei 450 nm mit einem Mikrotiterplatten Lesegerät **innerhalb von 15 Minunten** bestimmen.

7 BERECHNUNG DER ERGEBNISSE

Die T4-Konzentration der Proben wird wie folgt berechnet:

Eine Standardkurve wird erstellt, indem man die durchschnittliche Extinktion (y-Achse) der Referenzstandards gegen die entsprechende Konzentration in µg/dl (x-Achse) auf linearem Millimeterpapier aufträgt.

Die T4-Konzentration der Patientenprobe kann nun durch Interpolation aus dieser Standardkurve ermittelt werden. Wenn eine Reader-PC-Kombination vorhanden ist, kann die Berechnung der Daten per Computer erfolgen.

7.1 Beispiel einer Standardkurve

Die folgenden Daten dienen ausschließlich zur Demonstration und können keinesfalls zur Auswertung von Testergebnissen verwendet werden.

Total T4 (µg/dL)	Extinktion (450 nm)
0	2.667
2	1.786
5	1.060
10	0.778
15	0.591
25	0.384

8 NORMALWERTE

Der Normalwertbereich liegt bei 5,0 – 130 µg/dL.

9 SENSITIVITÄT

Die kleinste mit dem T4 ELISA nachweisbare T4-Konzentration beträgt 0.5 µg/dL.

Weitere Angaben zum Test entnehmen Sie bitte der englischen Arbeitsanleitung.

1 APPLICABILITÀ

Per la determinazione quantitativa della concentrazione totale di tiroxina (T4) nel siero umano.

2 INTRODUZIONE

L-tiroxina è un ormone sintetizzato e magazzinato nella ghiandola tiroidale. La proteolisi provocata dalla tiroglobulina follicolare provoca il rilascio di T4 nel sangue. Più del 99% di T4 è legato reversibilmente a tre proteine plasmatiche nel sangue – la globulina legante tiroxina (TGB) lega 70%, tiroxina legante albumina (TBA) lega 20% e l'albumina lega 10%. Circa 0.03% di T4 si trova permanentemente allo stato libero, non legato nel sangue.

Malattie che coinvolgano la funzione tiroidale possono presentare un ampio spettro di sintomi. La determinazione della concentrazione T4 attraverso test immunologici è il test più conveniente e affidabile per rilevare disfunzioni tiroidali. Concentrazioni innalzate di T4 si trovano durante l'ipertiroidismo causato dalla sindrome di Grave e di Plummer e in casi di acuto e subacuto tiroidismo. Conzentrazioni bassi di T4 sono associate con l'ipotiroidismo congenitale, mixedema, tiroidismo cronico (sindrome di Hashimoto) e con alcune anormalità genetiche.

3 PRINCIPIO DEL TEST

Nel test T4 ELISA i micropozzetti sono ricoperti di anticorpi. Determinati volumi dei campioni di siero, di anticorpo anti-T4 e un volume fisso di T4 coniugato alla perossidasi di rafano sono mescolati in ogni pozzetto. Durante l'incubazione, T4 e il T4 coniugato competono per il limitato numero di siti di legami sull'anticorpo. Dopo 60 minuti a temperatura ambiente i pozzetti sono lavati 5 volte con acqua per rimuovere il coniugato T4 non legato. Una soluzione TMB (benzidine tetrametilico) viene aggiunto e incubato per 20 minuti. La reazione enzimatica sviluppa un colore blu. La reazione è fermata dall'aggiunta di 1N HCl e l'assorbanza è misurata spettrofotometricamente a 450 nm. L'intensità del colore è direttamente proporzionale alla concentrazione di enzima presente e inversamente correlata alla quantità di T4 libero nel campione. Tramite una serie di T4 standard testati insieme ai campioni, la concentrazione ignota di T4 nei campioni può essere calcolata.

4 MATERIALI CONTENUTI NEL KIT

1. **Antibody-Coated Wells**
   Micropozzetti ricoperti con anti-T4 anticorpi di pecora.
2. **Enzyme Conjugate Concentrate** 11X
   Coniugato enzimatico concentrato (11 x), 1.3 mL.
3. **Enzyme Conjugate Diluent**
   Diluente del coniugato enzimatico, 13 mL
4. Reference **Standard** Set
   T4 standard di riferimento: 0, 2, 5, 10, 15, and 25 µg/dL,
   1 set, 1.0 mL ciascuno, pronto all'uso.
5. Reference **Standard** Set
   TMB, 11 mL
6. **Stop Solution**
   Soluzione d'arresto (1N HCl), 11 mL

5 MATERIALI RICHIESTI MA NON CONTENUTI NEL KIT

1. Acqua distillata.
2. Pipette a precisione: 25 µL, 100 µL, 200 µL, e 1.0 mL
3. Punte monouso.
4. Spettrofotometro per micropozzetti
5. Carta assorbente.
6. Carta millimetrata.
7. Agitatore vortex.

6 MAGAZZINAGGIO DEI REAGENTI

Test kits non aperti dovrebbero essere magazzinati a 2 °C - 8 °C. La data di scadenza è indicata sull'etichetta del kit.

7 COLLEZIONE DEI CAMPIONI

Per questo test dovrebbero essere utilizzati sieri senza aggiunte.

Prelevare il sangue tramite puntura venale, lasciare coagulare e separare il siero centrifugando il campione a temperatura ambiente.

8 PREPARAZIONE DEI REAGENTI

1. Portare tutti i reagenti a temperatura ambiente (18 °C - 25 °C) prima dell'uso.
2. **Preparazione della soluzione del coniugato enzimatico:**
   Diluizione 1+10:
   100 µL T4 coniugato + 1 mL con l'apposita soluzione di diluizione. Mescolare bene.
   Nota bene: Preparare soltanto la quantità necessaria per il kit.
   Il coniugato diluito dovrebbe essere utilizzato entro 24 ore.

9 ATTUAZIONE DEL TEST

1. Posizionare il numero necessario di pozzetti nell'aposito supporto.
2. Pipettare **25 µL** degli *standard, dei controlli e dei campioni* in ciascun pozzetto.
3. Aggiungere1**00 µL** *coniugato enzimatico* diluito in ogni pozzetto.
4. Mescolare agitando per almeno 30 secondi. Un completo mescolamento dei reagenti è molto importante!
5. Incubare per 60 minuti a temperatura ambiente (18 °C - 25 °C)
6. Vuotare i pozzetti capovolgendoli.
7. Lavare i pozzetti 5 volte con acqua distillata.
8. Dopo l'ultimo lavaggio rimuovere le rimanenti gocce d'acqua scuotendo i pozzetti contro la carta assorbente. (non utilizzare acqua del rubinetto!)
9. Aggiungere **100 µL** *soluzione TMB* in ogni pozzetto. Agitare cautamente per 5 secondi.
10. Incubare al buio a temperatura ambiente per 20 minuti.
11. Terminare la reazione con l'aggiunta di **100 µL** della *soluzione d'arresto*.
12. Mescolare cautamente per 30 secondi. **È importante che il colore blu vira completamente al giallo**.
13. Determinare l'estinzione a 450 nm con un lettore di micropozzetti **entro 15 minti**.

10 CALCOLO DEI RISULTATI

La concentrazione di T4 viene calcolata come segue:

Si costruisce una curva standard con le medie della estinzione in ordinata (y) degli standard e delle rispettive concentrazioni in µg/dL sull'ascisse (x) su carta linear-lineare o semi logaritmica.

La concentrazione di T4 dei campioni può essere determinata per l'interpolazione con la curva standard. Il calcolo può essere esegiuto anche al calcolatore.

10.1 Curva standard esemplare

I seguenti dati servono esclusivamente per la dimostrazione e non possono essere utilizzati in alcun caso per l'analisi dei risultati.

Total T4 (µg/dL)	Estinzione (DO) (450 nm)
0	2.667
2	1.786
5	1.060
10	0.778
15	0.591
25	0.384

11 VALORI NORMALI

I valori normali stanno tra 5,0 e 13,0 µg/dL.

12 SENSITIVITÀ

La concentrazione minima rilevabile con il test T4 ELISA è 0,5 µg/dL.

Per dettagli più precisi consultare la metodica in inglese.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	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
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	No. di Cat.	No de catálogo	Référence
LOT	Batch code *	Chargencode *	Lotto no	Número de lote	No. 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 conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Conditionnement
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité