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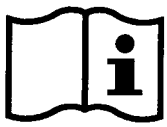

User´s Manual

Free Triiodothyronine (FT3) ELISA

Enzyme Immunoassay for the quantitative determination of Free Triiodothyronine (fT3) in human serum

IVD

CE

REF **DE2385**

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Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco 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 Only

Store at 2 °C to 8 °C.

1 INTENDED USE

For the quantitative determination of Free Triiodothyronine (fT3) concentration in human Serum.

2 INTRODUCTION

L-Triiodothyronine, a thyroid hormone, circulates in blood almost completely bound (>99.5%) to carrier proteins. The main transport protein is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of triiodothyronine is believed to be responsible for the biological action. Furthermore, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In individuals with normal thyroid function, as the concentrations of the carrier proteins change, the total T3 levels change in consert so that the free thriiodothyronine (fT3) concentration remains constant. Thus, measurements of fT3 concentrations correlate more reliably with clinical status than total triiodothyronine levels.

For example, the increase in total triiodothyronine levels associated with pregnancy, oral contraceptives, and estrogen therapy result in higher total T3 levels while the fT3 concentration remains basically unchanged.

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations in a direct determination of fT3

3 PRINCIPLE OF THE TEST

The fT3 test is a solid phase competitive enzyme immunoassay. Patient serum samples, standards, and T3-Enzyme Conjugate Working Reagent is added to wells coated with monoclonal T3 antibody. fT3 in the patient specimen and the T3 labeled conjugate compete for available binding sites on the antibody. After a 60 minutes incubation at room temperature, the wells are washed with water to remove unbound T3 conjugate. A solution of H_2O_2/TMB is then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 3N 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 fT3 in the sample. By reference to a series of fT3 standards assayed in the same way, the concentration of fT3 in the unknown sample is quantified.

4 REAGENTS

4.1 Materials provided with the kit

- **AntibodyCoated Microplate** (1 plate, 96 wells)
  Microtiter wells coated with Anti-T3
- fT3-**Enzyme Conjugate** Reagent, ready to use (1 vial, 10.5 ml)
  Contains T3 Ab conjugated to horseradish peroxidase with preservatives
- Free T3 Reference **Standard** Set (1.0 ml/vial)
  Contains 0, 0.9, 2.2, 5.0, 9.0 and 19.0 pg/ml of fT3 in human serum with preservatives; liquid, ready to use
  ** Exact levels are given on the labels on a lot specific basis*
- **Color Reagent A**, (1 bottle, 13 ml)
  Contains hydrogen peroxide in acetate buffer
- **Color Reagent B** (1 bottle, 13 ml)
  Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
- **Stop Solution** (3N HCl) (1 bottle, 10 ml)
  Contains diluted hydrochloric acid

4.2 Materials required but not provided:

- Pipette capable of delivering 50 µl volumes with a precision of better than 1.5%.
- Dispenser(s) for repetitive deliveries of 0.050 ml and 0.200 ml volumes with a precision of better than 1.5%.
- Microplate Reader with 450 nm wavelength absorbance capability.
- Test tubes for dilution of enzyme conjugate and for mixing Color Reagent A with Color Reagent B.
- Absorbent paper of blotting the microplate wells.
- Timer.
- Quality control materials.

5 SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques.

This kit is for use with serum sample without additives only.

Serum samples may be refrigerated at 2°C - 8°C for a maximum period of 48 hours. If the samples can not be assayed within 48 hours, they may be stored at temperatures of –20°C for up to 30 days.

6 STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2°C - 8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air.

Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.

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

7 REAGENT PREPARATION

Working Substrate Solution – Prepare immediately before use.

To prepare H_2O_2/TMB solution, make a 1:1 mixing of Color Reagent A with Color reagent B up to 1 hour before use. Mix gently to ensure complete mixing.

The prepared H_2O_2/TMB reagent should be made at least 15 minutes before use and is stable at room temperature in the dark for up to 3 hours. Discard excess after use.

8 ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18°C – 25°C).

1. Format the microplates' wells for each serum reference, control, and patient specimen to be assayed in duplicate.
2. Pipette 0.050 ml (50 µl) of the appropriate serum reference, control, and specimen into the assigned well.
3. Add 0.100 ml (100 µl) of T3-Enzyme Conjugate Solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 0.200 ml (200 µl) of **Working Substrate Solution** to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.** Gently mix for 10 seconds.
8. Incubate at room temperature in the dark for 20 minutes.
9. Stop the reaction by adding 50 µl of 3N HCl to each well.
10. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely**.
11. Read absorbance at 450 nm with a microtiter well reader within 30 minutes.

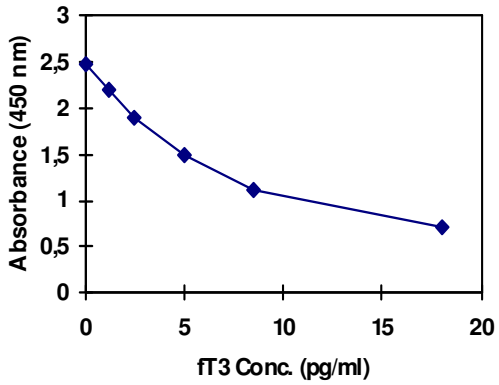
9 CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in pg/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of fT3 in pg/ml from the standard curve.

9.1 Example of Standard Curve

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against fT3 concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

fT3 (ρg/ml)	Absorbance (450 nm)
0	2.474
1.2	2.202
2.5	1.884
5.0	1.485
8.5	1.117
18.0	0.710



10 PERFORMANCE CHARACTERISTICS

10.1 Accuracy

The fT3 Microplate EIA Test System was compared with a coated tube radioimmunoassay method. Biological specimens from hypothyroid, euthyroid, and hyperthyroid populations were used (Values ranged from 0.1 pg/ml – 14 pg/ml). The total number of such specimens was 151.

The least square regression equation and the correlation coefficient were computed for this fT3 EIA Test System in comparison with the reference method. The data obtained is shown in the following table:

Method	Mean (X)	Least Square Regression Analysis	Coefficient
This method	3.045	y = 0.978(x) - 0.116	0.950
Reference	2.921		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

10.2 Precision

The within and between assay precision of the fT3 Microplate EIA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are shown in the following tables:

Within Assay Precision (Values in pg/ml)

Sample	N	X	S.D.	C.V %.
Low	24	1.85	0.09	4.9
Normal	24	4.49	0.16	3.6
High	24	8.0	0.25	3.1

Between Assay Precision (Values in pg/ml)*

Sample	N	X	S.D.	C.V. %
Low	12	2.16	0.29	13.1
Normal	12	5.09	0.40	7.9
High	12	9.13	0.94	10.2

*As measured in ten experiments in duplicate over a ten day period.

10.3 Specificity

The cross-reactivity of the triiodothyronine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ration between dose of interfering substance to dose of Triiodothyronine needed to displace the same amount of tracer.

Substance	Cross Reactivity	Concentration
I-Triiodothyronine	1.0000	-
I-Thyroxine	< 0.0002	10 µg/mL
Iodothyrosine	< 0.0001	10 µg/mL
Diiodothyrosine	< 0.0001	10 µg/mL
Phenylbutzone	< 0.0001	10 µg/mL
Sodium Salicylate	< 0.0001	10 µg/mL

10.4 Sensitivity

The fT3 EIA procedure has a sensitivity of 0.05 pg/ml. The sensitivity was ascertained by determining the variability of the 0 pg/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

11 EXPECTED VALUES

A study of euthyroid adult population was undertaken to determine expected values for the fT3 EIA Test System. The mean (X) values, standard deviations (σ) and expected ranges (± 2σ) are presented in the following table:

Expected Values for the Free T3 ELISA (in pg/ml)

	Adult (110 specimens)	**Pregnancy (75 specimens)**
Mean (X)	2.8	3.0
Standard Deviation (σ)	0.7	0.6
Expected Ranges (± 2 σ)	1.4 - 4.2	1.8 - 4.2

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of “normal” persons is dependent upon several factors: the specificity of the method, the population tested, and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

12 CLINICAL SIGNIFICANCE

Alterations in the concentration of serum binding proteins will generally result in a corresponding change in total T3 concentrations while the physiologically active fT3 level remains largely unchanged in an euthyroid individual. Therefore, determination of fT3 concentration may provide a more accurate assessment of thyroid status than total T3 measurement. Elevated fT3 Concentrations are indicative of hyperthyroidism and low levels are indicative of hypothyroidism.

13 LIMITATIONS OF THE PROCEDURE

1. 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.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. 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.

14 REFERENCES/LITERATURE

1. Tietz, N.W., Fundamentals of Clinical Chemistry, 2nd Ed., pg. 602, Sauders Press, Phila., 1976.
2. Horworth, P.J.N., Ward, RL., J. Clin Pathol. 1972; 25:259-62.
3. Sati, C., Chattor, A.J., Watts, N. Fundamentals of Clinical Chemistry. Ed. Tietz, N.W. 3rd Ed., pg. 586. Saunders press Phila. 1987.
4. Lundberg, P.A., Jagenburg, R., Lindstedt, G., Nystrom, E., Clin. Chem. 1982, 28:1241.
5. Melmed, S., Geola, F.L., Reed, A.W., Pekary, A.E., Park, J., Hershmen, J. M., Clin Endocrin. Metabol. 1982, 54;300.
6. Ingbar, S.H., et al. J. Clin. Invest., 1965, 44:1679.
7. Selenkow, H.A., and Robin, N.I., J. Maine Med. Assoc. 1970, 61:199.
8. Oppenheimer, J.H., et al. J. Clin. Invest. 1962, 42: 1769.
9. Dick, M., Watson, F., Med J. Aust. 1980, 1:115.
10. Dussault, j. H., Turcotte, R., and Gieyda, H., Clin Endocrin. Metabol. 1976, 43: 232-285.
11. Tarnoky, A.L. Advan. Clin. Chem. 1981, 21:101-146.
12. Emrich, D., Schondube, H.,- Sehlen, S., and Schreivagel, I., Nuc. Compact, 1985, 16:392.
13. Procedures for Decontamination of Plumbing Systems Containing Copper and/or Lead azides, Dept. of H.E.W., N.I.O.S.H., Rockville, Maryland, 1976.

1 VERWENDUNGSZWECK

Enzymimmunoassay für die quantitative Bestimmung von Freier Triiodothyronin Konzentration in humanem Serum.

2 TESTPRINZIP

Der FT3 ELISA ist ein Festphasen-Enzymimmunoassay, der auf dem Prinzip der kompetitiven Bindung basiert.

3 REAGENZIEN

3.1 Im Kit enthalten

1. **Coated Microplate:** beschichtete Mikrotiterplatte, 96 Vertiefungen
2. **Enzyme Conjugate:** 1 Fläschchen mit T3-Enzymkonjugat, 10,5 mL. Gebrauchsfertig
3. **Standards**: 6 Fläschchen
   1.0 mL/Fläschchen, gebrauchsfertig.
   Die lot-abhängigen Konzentrationen stehen auf dem Fläschchenetikett.
4. **Color Ragent A:** Eine Flasche Substrat A. 13 mL
5. **Color Reagent B:** Eine Flasche Substrat B. 13 mL
6. **Stop Solution:** Eine Flasche Stopplösung, (3N HCl). 10 mL

3.2 Benötigte Materialien die nicht im Kit enthalten sind:

- Präzisions-Pipetten: 50 µl, 20 µL - 200 µL und 200 µL - 1.0 mL; Einmal Pipettenspitzen
- Microtiter Platten Lesegerät (450nm Wellenlänge)
- Destilliertes Wasser
- Gefäße zum Mischen der Substratlösung A und B
- Saugpapier oder Papierhandtuch
- Laborwecker
- Qualitätskontrollmaterial

4 PROBEN

Blut durch Venenpunktion entnehmen, gerinnen lassen und das Serum durch Zentrifugation bei Raumtemperatur abtrennen. Für diesen Test sollten nur Serumproben ohne Zusätze verwendet werden.

Serumproben können bei 2-8°C für maximal 48 Stunden gelagert werden. Tiefgefroren bei –20°C ist eine Lagerung für bis zu 30 Tagen möglich.

5 AUFBEWAHRUNG DER REAGENZIEN

Die ungeöffneten Reagenzien behalten bei Lagerung um 2-8°C ihre Reaktivität bis zum Verfallsdatum. Nach dem Verfallsdatum die Reagenzien nicht mehr verwenden.

Nach dem Öffnen sollten alle Reagenzien bei 2 8°C gelagert werden.

Die Mikrotiterwells sollten bei 2 8°C gelagert werden. Der einmal geöffnete Folienbeutel sollte stets sehr sorgfältig wieder verschlossen werden. Unter den beschriebenen Lagerbedingungen behalten geöffnete Kits ihre Reaktivität bis zum angegebenen Verfallsdatum

6 VORBEREITUNG DER REAGENZIEN

Herstellung der **gebrauchsfertigen Substratlösung**:
Mischen Sie zu gleichen Teilen Substrat A und Substart B bis zu 60 Minuten vor der Durchführung des Assays. Z.B. für ein 8-Well Streifen benötigen Sie 1 mL Substrat A und 1 mL Substrat B. Mischen Sie die Lösung gründlich. Die gebrauchsfertige Lösung ist im Dunkeln bei Raumtemperatur 3 Stunden haltbar. Verwerfen Sie evtl. anfallende Reste.

7 TESTDURCHFÜHRUNG

Alle Reagenzien sowie die benötigte Anzahl von Wells sollen vor dem Gebrauch auf Raumtemperatur gebracht werden.

1. Die benötigte Anzahl an Wells in der Halterung befestigen.
2. 50 µl Standards, Kontrollen und Proben in die entsprechenden Vertiefungen pipettieren.
3. 100 µl gebrauchsfertiges Enzymkonjugat in alle Vertiefungen geben.
4. Ca. 20-30 Sek. gründlich mischen. **Eine vollständige Vermischung der Reagenzien ist sehr wichtig!**
5. 60 Min. bei Raumtemperatur (18-25°C) inkubieren.
6. Inkubationslösung zügig abschütten. Vertiefungen 5-mal mit Aqua dest., ca. 300 µL pro Well, waschen. Nach dem letzten Waschen Wassertropfen aus den Vertiefungen durch Ausklopfen auf saugfähigem Papier entfernen. (Bitte kein Leitungswasser verwenden!)
7. 200µl Substratlösung in alle Vertiefungen geben. (Siehe Kap.6) Vorsichtig für 10 Sekunden mischen.
8. 20 Minuten bei Raumtemperatur im Dunkeln inkubieren.
9. Die Reaktion durch Zugabe von 50 µL Stopplösung stoppen.
10. 30 Sek. vorsichtig mischen. **Es ist wichtig, dass der Farbumschlag von Blau zu Gelb vollständig erfolgt.**
11. Die Extinktion bei 450 nm mit einem Mikrotiterplatten-Lesegerät innerhalb von 30 Min. bestimmen.

8 BERECHNUNG DER ERGEBNISSE.

Die F T3-Konzentration der Proben wird wie folgt berechnet:

Eine Standardkurve wird erstellt, indem man die durchschnittl. Extinktion (y) der Referenzstandards gegen die entsprechende Konzentration in pg/ml (x) auf linearem Millimeterpapier aufträgt.

Die FT3-Konzentration der Patientenprobe kann nun durch Interpolation aus dieser Standardkurve ermittelt werden. Falls vorhanden, kann die Berechnung der Daten mit entsprechender Software per Computer erfolgen.

8.1 Beispielhafte Standardkurve

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

FT3 (pg/ml)	Absorbance (450 nm)
0	2.474
1.2	2.202
2.5	1.884
5.0	1.485
8.5	1.117
18.0	0.710

9 TEST-CHARAKTERISITKA

Sensitivität

Die kleinste mit dem FT3 ELISA nachweisbare F T3-Konzentration beträgt 0.05 pg/mL.

Weitere Daten entnehmen sie bitte der ausführlichen englischen Anleitung.

10 ERWARTETE WERTE

Es wird empfohlen, dass jedes Labor seine eigenen normalen und abnormalen Werte ermittelt.

	Erwachsene (pg/ml)	**Schwangere (pg/ml)**
Mittelwert (X)	2.8	3.0
Standard Abweichung (S.D.)	0.7	0.6
Erwarteter Bereich (± 2 S.D.)	1.4 - 4.2	1.8 - 4.2

11 GRENZEN DES TESTS

Jede unsachgemäße Behandlung von Proben oder Modifikationen dieses Tests können die Ergebnisse beeinflussen.

1. Der Test muss exakt gemäß der Testanleitung des Herstellers abgearbeitet werden. Darüber hinaus muss der Benutzer sich strikt an die Regeln der GLP (Good Laboratory Practice) oder andere eventuell anzuwendende Regeln oder nationale gesetzliche Vorgaben halten.
2. Die Sensitivität und Präzision dieses Assays wird erheblich beeinflusst von der korrekten Durchführung des Waschschrittes!
3. Lipämische, ikterische und/oder stark hämolysierte Proben sollten nicht verwendet werden.
4. Das Testergebnis allein sollte niemals als alleinige Grundlage für die Einleitung therapeutischer Konsequenzen dienen.

1 INTRODUZIONE

1.1 Applicabilità

Per la determinazione quantitativa di triiodiotironina (fT3) nel siero umano.

1.2 Introduzione

Triiodiotironina, un ormone tiroidale, circola nel sangue quasi completamente (>99.5%) legato a proteine trasportatori (1,2). La più importante proteina tra queste è la globulina tiroxina-legante (TBG). Soltanto la parte non legata (libera) della triiodiotironina sembra essere biologicamente attiva. Inoltre, le concentrazioni delle proteine trasportatrici possono variare a seconda della condizione clinica del paziente, per esempio durante la gravidanza. In uno stato di normale funzionalità tiroidale la concentrazione totale della triiodiotironina rimane costante, anche se la concentrazione delle proteine carrier varia. Perciò la determinazione della triiodiotironina libera rispecchia più accuratamente lo stato clinico.

Per esempio, l'aumento dei livelli della triiodiotironina totale legato alla gravidanza o alla terapia contracettiva orale o di estrogeni porta a valori alzati della T3 totale, mentre la concentrazione della T3 libera rimane praticamente inalterata.

La metodologia a base di un immunoassay enzimatico su micropiastre garantisce una diretta determinazione di T3 libera con un ottima sensitività e un minimo a manipolazioni tecniche da parte dell'operatore.

2 PRINCIPIO DEL TEST

Il fT3 test è un immunoassay enzimatico competitivo in fase solida. Campioni di siero, standard e il coniugato enzimatico T-3 sono mescolati in pozzetti ricoperti con l'anticorpo monoclonale T3. fT3 nel siero dei pazienti e il coniugato competono per i siti di legame dell'anticorpo. Dopo 60 minuti di incubazione a temperatura ambiente i pozzetti sono lavati con acqua per rimuovere il coniugato T3 non legato. Una soluzione di H_2O_2 /TMB (benzidine tetrametilico) viene aggiunto e incubato per 20 minuti. Il risultato è lo sviluppo di un colore blu. La reazione è fermata dall'aggiunta di 3 N 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 fT3 libero nel campione. Tramite una serie di fT3 standard testati insieme ai campioni, la concentrazione ignota di fT3 nei campioni può essere calcolata.

3 REAGENTI

3.1 Materiali contenuti nel kit:

1. **Coated Microplate:** Micropozzetti a 96 pozzetti ricoperti con anticorpi
2. **Enzyme Conjugate:** Un (1) flacone con coniugato enzimatico T3; 10.5 mL, pronto all'uso
3. **Standard**: Sei (6) flaconi con standards di triiodiotironina,
   1.0 mL / flacone, pronto all'uso
   I valori sono indicati sull'etichetta dei flaconi.
4. **Color Reagent A**: Un (1) flacone di substrato A, 13 mL
5. **Color Reagent B:** Un (1) flacone di substrato B, 13 mL
6. **Stop Solution**: Un (1) flacone di soluzione d'arresto, 3 N HCl, 10 mL

3.2 Materiali richiesti ma non contenuti nel kit:

- Pipette a precisione: 50 µl, 20 µL - 200 µL und 200 µL - 1.0 mL
- Spettrofotometro per micropozzetti (450nm lunghezza d'onda)
- Sciacquatore per micropozzetti
- Acqua distillata
- Contenitori per mescolare le soluzioni substrato A e B.
- Agitatore vortex
- Materiale per il controllo qualità

4 COLLEZIONE DEI CAMPIONI

Prelevare il sangue tramite puntura venale, lasciare coagulare e separare il siero centrifugando il campione a temperatura ambiente. Per questo test dovrebbero essere utilizzati sieri senza aggiunte.

Campioni di sieri possono essere magazzinati per 48 ore a 2-8°C. Per un magazzinaggio fino a 30 giorni i campioni dovrebbero essere congelati a –20°C.

5 MAGAZZINAGGIO DEI REAGENTI

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

A 2-8°C i reagenti non aperti rimangono reattivi fino alla data di scadenza indicata. Non usare reagenti oltre questa data.

Tutti i reagenti aperti devono essere magazzinati a 2-8°C. I micropozzetti devono essere magazzinati a 2-8°C. Una volta aperti i pacchi, questi devono essere richiusi accuratamente.

6 PREPARAZIONE DEI REAGENTI

Preparazione della **soluzione substrato**:

Mescolare parti uguali di substrato A e substrato B fino a 15 minuti prima dell' attuazione del test. Per una colonna di 8 pozzetti si necessitano 1 mL di substrato A e 1 mL di substrato B. Mescolare accuratamente la soluzione. La soluzione pronta all'uso si mantiene per 3 ore al buio. Soluzioni avanzate devono essere eliminate.

7 ATTUAZIONE DEL TEST

Tutti i reagenti e i campioni devono essere portati a temperatura ambiente e ben mescolati prima dell'uso.

1. Posizionare il numero necessario di pozzetti nell'apposito supporto.
2. Pipettare 50 µL degli standard, dei controlli e dei campioni in ciascun pozzetto.
3. Aggiungere100 µL coniugato enzimatico pronto all'uso in ogni pozzetto.
4. Mescolare agitando per 20-30 secondi. **Un completo mescolamento dei reagenti è molto importante!**
5. Incubare per 60 minuti a temperatura ambiente (18-25°C).
6. Vuotare i pozzetti capovolgendoli. Lavare i pozzetti 5 volte con acqua distillata, ca 300 µL per pozzetto. Dopo l'ultimo lavaggio rimuovere le rimanenti gocce d'acqua scuotendo i pozzetti contro la carta assorbente. (non utilizzare acqua del rubinetto!)
7. Aggiungere 200 µL soluzione substrato in ogni pozzetto.
8. Coprire e incubare al buio a temperatura ambiente per 20 minuti.
9. Terminare la reazione con l'aggiunta di 50 µL della soluzione d'arresto.
10. Mescolare cautamente per 30 secondi. **È importante che il colore blu vira completamente al giallo.**
11. Determinare l'estinzione a 450 nm con un lettore di micropozzetti **entro 30 min.**

8 CALCOLO DEI RISULTATI

La concentrazione di fT3 viene calcolata come segue:

Si costruisce una curva standard con le medie della estinzione in ordinata (y) degli standard e delle rispettive concentrazioni in pg/mL sull'ascisse (x).

La concentrazione di fT3 dei campioni può essere determinata per interpolazione con la curva standard. Il calcolo può essere esegiuto anche al calcolatore.

8.1 Curva standard esemplare

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

FT3 (pg/ml)	Assorbanza (450 nm)
0	2.474
1.2	2.202
2.5	1.884
5.0	1.485
8.5	1.117
18.0	0.710

9 CARATTERISTICHE DEL TEST

Sensitività

La concentrazione minima rilevabile con il test fT3 ELISA è 0.05 pg/ml.

Per dettagli più precisi consultare la metodica in inglese.

10 VALORI NORMALI

È consigliabile che ogni laboratorio determini i propri valori normali e anormali.

	adulti (pg/mL)	Donne gravide (pg/mL)
Valori medi (X)	2.8	3.0
Deviazione standard (S.D.)	0.7	0.6
Deviazione st. allargata (±2 S.D.)	1.4 - 4.2	1.8 - 4.2

11 LIMITAZIONE DEL TEST

Ogni manutenzione impropria dei campioni o modificazione del protocollo può influenzare I risultati.

1. Il test deve essere eseguito esattamente secondo il protocollo dato dal produttore. Inoltre l'utente deve seguire le regole del GLP (Good Laboratory Practice) o eventualmente altre regole comportamentali o disposizioni legali.
2. Il lavaggio è un passaggio critico. Lavaggio insufficiente o la mancata rimozione d'acqua dopo i lavaggi porta a una precisione minore e a estinzioni falsamente alti.
3. Non usare campioni emolitici, itterici o lipemici.
4. Il risultato del test da solo non è base sufficiente per lo stabilimento di una terapia.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..