

Product information



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User's Manual

T3 free ELISA

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



IB79136R



96 wells



For Research Use Only – Not for Use in Diagnostic Procedures

Please use only the valid version of the Instructions for Use provided with the kit.

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Store at 2 °C to 8 °C.

1 INTENDED USE

For the determination of Free Triiodothyronine (fT3) concentration in human serum.
For research use only – Not for use in diagnostic procedures.

2 PRINCIPLE OF THE TEST

The fT3 test is a solid phase competitive enzyme immunoassay. Serum samples, standards, and T3-Enzyme Conjugate Working Reagent is added to wells coated with monoclonal T3 antibody. fT3 in the sample 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 H₂O₂/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.

3 REAGENTS

3.1 Materials provided with the kit

- SORB** **MT** **Antibody Coated Microplate** (1 plate, 96 wells) Microtiter wells coated with Anti-T3
- ENZ** **CONJ** **fT3-Enzyme Conjugate** Reagent, ready to use (1 vial, 10.5 mL) Contains T3 Ab conjugated to horseradish peroxidase with preservatives
- CAL** **1** – **6** **Free T3 Reference Standard Set** (1.0 mL/vial) Contains 0 - 0.9 - 2.2 - 5.0 - 9.0 - 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** **REAG** **A** **Color Reagent A**, (1 bottle, 13 mL) Contains hydrogen peroxide in acetate buffer
- COLOR** **REAG** **B** **Color Reagent B** (1 bottle, 13 mL) Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
- STOP** **SOLN** **Stop Solution** (3N HCl) (1 bottle, 10 mL) Contains diluted hydrochloric acid

3.2 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.

4 SAMPLE COLLECTION AND PREPARATION

Serum should be prepared from a whole blood sample obtained by acceptable medical techniques. This kit is for use with serum sample without additives only. Serum samples may be refrigerated at 2-8 °C for a maximum period of 48 hours. If the samples cannot be assayed within 48 hours, they may be stored at temperatures of -20 °C for up to 30 days.

5 STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8 °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.

6 REAGENT PREPARATION

Working Substrate Solution – Prepare immediately before use.

To prepare H₂O₂/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 H₂O₂/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.

7 ASSAY PROCEDURE

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

1. Format the microplates' wells for each serum reference, control, and samples to be assayed in duplicate.
2. Pipette 0.050 mL (50 µL) of the appropriate serum reference, control, and samples 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 deionized 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.

8 RESULTS

1. Calculate the mean absorbance value (A_{450}) for each 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 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 sample to determine the corresponding concentration of fT3 in pg/mL from the standard curve.

9 PERFORMANCE CHARACTERISTICS

9.1 Accuracy

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

9.2 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.

9.3 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

9.4 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.

10 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. For research use only.

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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Français	Espanol	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
<i>Distributed by</i>	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore