Free Thyroxine (fT4) ELISA

For the quantitative determination of free thyroxine (fT4) in human serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 25-FT4HU-E01
Size: 96 Wells
Version: 19.1 2023/06 – ALPCO 2.0

INTENDED USE

The Free Thyroxine ELISA is an enzyme immunoassay for the quantitative determination of free thyroxine (fT4) in human serum. For research use only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The Free Thyroxine ELISA is a solid phase competitive enzyme immunoassay. Serum samples, standards, and Thyroxine-Enzyme Conjugate Working Reagent are added to wells coated with monoclonal thyroxine antibody. Free thyroxine in the samples and the thyroxine-labeled conjugate compete for available binding sites on the antibody. After a 60 minute incubation at room temperature, the wells are washed with water to remove unbound thyroxine 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 free thyroxine in the sample. By reference to a series of free thyroxine standards assayed in the same way, the concentration of free thyroxine in the unknown sample is quantified.

Reagents

Materials provided with the kit:

- Antibody Coated Microplate, 96 wells – coated with Anti-T4
- fT4-Enzyme Conjugate Reagent, ready to use, 1 vial, 10.5 mL – contains T4 Ab conjugated to horseradish peroxidase with preservatives
- Free T4 Reference Standard Set – Six vials of serum reference for fT4 at approximate* concentrations of 0, 0.4, 1.1, 2.2, 4.1, and 8.0 ng/dL, a preservative has been added; liquid, ready to use,1 mL each

  **Exact levels are given on the labels on a lot specific basis*

  For SI units 1ng/dL x 12.9 = pmol/L
- 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 a buffer solution
- Stop Solution (3N HCl), 1 bottle, 10 mL – contains diluted hydrochloric acid

Materials required but not provided

- Pipette capable of delivery 50 µL volumes with a precision greater than 1.5%
- Dispenser(s) for repetitive deliveries of 50 µL and 200 µL volumes with a precision of better than 1.5%
- Microplate Reader with 450 nm wavelength absorbance capability
- Test tubes for mixing Color Reagent A with Color Reagent B
- Absorbent paper for blotting after washing the microplate
- Timer
- Quality control materials

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 desiccant to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided they are stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0 - 2.0 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Working Substrate Solution – Prepare immediately before use

To prepare H_2O_2/TMB solution, make a 1:1 mix of Color Reagent A with Color Reagent B up to 1 hour before use (or 3 hours if stored in the dark). 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 on the bench for up to 1 hour and in the dark for up to 3 hours. Discard excess after use.

SAMPLE COLLECTION AND PREPARATION

Serum should be prepared from a whole blood sample obtained by acceptable 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 -20 °C for up to 30 days.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum samples and controls (not included) to room temperature (18-25°C).

1. Format the microplate wells for each reference standard, control, and sample to be assayed in duplicate.
2. Pipette 0.05 mL (50 µL) of the appropriate reference standard, control, and sample into the assigned wells.
3. Add 0.100 mL (100 µL) of Free Thyroxine Enzyme Conjugate Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Incubate for 60 minutes at room temperature.
6. Remove the incubation mixture by emptying the plate contents 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 (Stop Solution) 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 plate reader within 30 minutes.

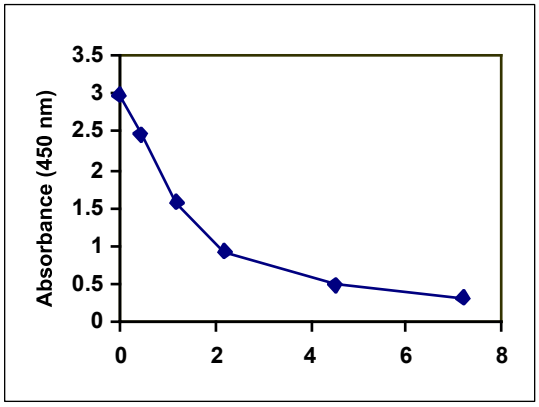
CALCULATION OF RESULTS

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 ng/dL 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 free thyroxine in ng/dL from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against free thyroxine 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.

fT4 (ng/dL)	Absorbance (450 nm)
0	2.797
0.45	2.465
1.2	1.582
2.2	0.934
4.5	0.487
7.2	0.320



ASSAY CHARACTERISTICS

Specificity (cross-reactivity)

The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thryroxine needed to displace the same amount of conjugate.

Cross-Reactant	% Cross-Reactivity	Concentration
l-Thyroxine (T4)	100	--
d-Thyroxine	98	10 µg/dL
l-Triiodothyronine (T3)	3.0	100 µg/dL
d-Triiodothyronine	1.5	100 µg/dL
Diiodothyronine	0.01	100 µg/mL
Diiodotyrosine	0.01	100 µg/mL
Iodotyrosine	0.01	100 µg/mL
Phenytoin	N/D	40 µg/mL
Sodium Salicylate	N/D	500 µg/mL
TBG	N/D	40 µg/mL
Albumin	N/D	40 µg/mL
Phenylbutazone	N/D	10 µg/mL

Analytical Sensitivity

The ft4 ELISA procedure has a detection limit of 0.05 ng/dL. The detection limit was ascertained by determining the variability of the 0 ng/dL serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

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 instructions for use 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. If the device fails to perform, contact ALPCO.