POINTE SCIENTIFIC, INC.
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Free Thyroxine (fT4) Enzyme Immunoassay Test Kit

Intended Use

For the quantitative determination of Free Thyroxine (fT4) concentration in human serum. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Principle

The fT4 test is a solid phase competitive enzyme immunoassay. Serum samples, standards, and Thyroxine-Enzyme Conjugate Working Reagent are added to wells coated with monoclonal T4 antibody. fT4 in the patient specimen and the T4 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 T4 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 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled fT4 in the sample. By reference to a series of fT4 standards assayed in the same way, the concentration of fT4 in the unknown sample is quantified.

Materials Provided

1. T4 Antibody-Coated Microplate, 96 wells
2. T4-Enzyme Conjugate Reagent, ready to use, 10.5ml
3. Free T4 Reference Standards, 0, 0.3, 0.95, 2.1, 3.6, and 7.0ng/dl**, 1 ml each.
   **Exact levels are given on the labels o a lot specific basis.
4. Color Reagent A, 13ml
5. Color Reagent B, 13ml
6. Stop Solution (3N HCl), 10ml

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 they are stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm 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 mixture 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.

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 specimen to be assayed in duplicate.
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control and specimen into the assigned well.
3. Add 0.100 ml (100µl) of Free T4 Enzyme Conjugate Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Incubate 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 distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 0.200 ml (200µl) 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 50µl 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 well reader within 30 minutes.

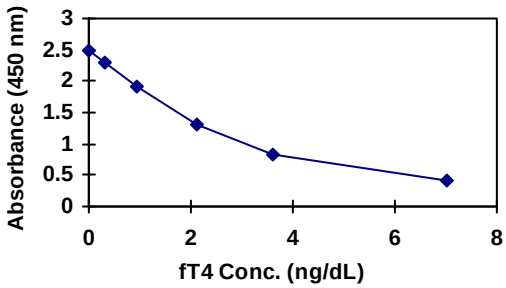
Calculations

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 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 specimen to determine the corresponding concentration of fT4 in ng/dl from the standard curve.

Example of Standard Curve

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against fT4 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 (450nm)
0	2.496
0.3	2.292
0.95	1.903
2.1	1.295
3.6	0.819
7.0	0.410



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