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Free Triiodothyronine (fT3) ELISA

Catalog No. F3376T (96 tests)

INTENDED USE

The Calbiotech, Inc. fT3 ELISA kit is used for the quantitative measurement of free Triiodothyronine (fT3) in human serum or plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Free triiodothyronine (FT3) is the active form of thyroid hormone, regulating metabolic rate, growth, and development. This assay detects FT3 for studies on thyroid physiology, receptor function, and endocrine regulation of metabolism.

PRINCIPLE OF THE TEST

The fT3 is a solid phase competitive ELISA. The samples, and fT3 enzyme conjugate are added to the wells coated with anti-T3 monoclonal antibody. fT3 in the patient's serum competes with T3 enzyme conjugate for binding sites in anti-T3 antibody. Unbound T3 and T3 enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of fT3 in the samples. A standard curve is prepared relating color intensity to the concentration of the fT3.

MATERIALS PROVIDED	96 TESTS
1. Microwell coated with fT3 MAb	12x8x1
2. fT3 Standard: 6 vials (ready to use)	0.5 mL
3. fT3 Control: 2 vials (ready to use)	0.5 mL
4. fT3 Enzyme conjugate: 1 bottle (ready to use)	12 mL
5. TMB Substrate: 1 bottle (ready to use)	12 mL
6. Stop Solution: 1 bottle (ready to use)	12 mL
7. 20X Wash Concentrate: 1 bottle	25 mL

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 – 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

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WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature.

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Format the microplates' wells for control, standard and patient samples to be assayed in duplicate. Place any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 50 µl of fT3 standards, control and samples into the assigned well.
3. Add 100 µl of fT3 enzyme conjugate to all wells.
4. Incubate for 60 minutes at room temperature (18-26° C).
5. Remove liquid from all wells. Fill wells with 300 µl 1X wash buffer (see buffer preparation above) Wash three times. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check fT3 standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit.
2. To construct the standard curve, plot the absorbance for fT3 standards (vertical axis) versus fT3 standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

EXAMPLE OF STANDARD CURVE

	OD 450 nm	Conc. pg/mL
Std 1	2.329	0
Std 2	1.803	2.5
Std 3	1.130	4
Std 4	0.390	7
Std 5	0.195	14
Std 6	0.091	22

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.