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Free Triiodothyronine (fT3) ELISA

Catalog No. IB19105N (96 Tests)

INTENDED USE

The fT3 ELISA kit is used for the quantitative measurement of free Triiodothyronine (fT3) in human serum or plasma. For research use only – Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

Over 99% of Triiodothyronine (T3) circulates in blood is bound to carrier proteins; thyroxine-binding globulin (TBG). However, only the free (unbound) portion of T3 is responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total T3 level changes so that the freeT3 concentration remains constant. Thus, measurements of free T3 concentrations correlate more reliably with clinical status than total T3 levels. The increase in total T3 levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T3 levels while the free T3 concentration remains basically unchanged.

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 serum competes with T3 enzyme conjugate for binding sites of the anti-T3 antibody. Unbound T3 and T3 enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of the 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. Microwells coated with fT3 Mab	12x8x1
2. fT3 Standard: 6 vials (ready to use)	0.5 ml
3. fT3 Enzyme conjugate: 1 Bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12 ml
5. Stop Solution: 1 bottle (ready to use)	12 ml
6. 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.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls 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, 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. This kit is designed for research use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which samples or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. 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.

SAMPLE COLLECTION HANDLING

1. Collect blood samples and separate the serum immediately.
2. Samples may be stored refrigerated at (2-8° C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic samples.

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

1. Format the microplates wells for control, standard and 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

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for fT3 were established by the CBI and may be used as initial guideline ranges only:

Classification	pg/ml
Adult	1.4-4.2

LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean (pg/mL)	Standard Deviation	Mean + SD (Sensivity)
Zero Standard	24	2.009	0.082	1.8454

2. Precision

Intra-Assay

Serum	No. of Replicates	Mean (pg/mL)	Standard Deviation	Coefficient of Variation (%)
1	16	1.862	0.180	9.68%
2	16	5.220	0.165	3.16%
3	16	7.721	0.409	5.29%

Inter-Assay

Serum	No. of Replicates	Mean (pg/mL)	Standard Deviation	Coefficient of Variation (%)
1	24	2.07	0.15	7.35%
2	24	5.15	0.13	2.56%
3	24	8.04	0.51	6.33%