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Product Information

Free Triiodothyronine (fT3) ELISA kit

Catalog Number: EA100839
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use
The fT3 ELISA kit is used for the quantitative measurement of free Triiodothyronine (fT3) in human serum.

Background
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 anti-T3 biotin and fT3 enzyme conjugate are added to the wells coated with Streptavidin. fT3 in the patient's serum competes with a T3 enzyme conjugate for binding sites. 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

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. fT3 Standard: 6 vials (ready to use)	0.5 ml
3. Anti-T3 Biotin Solution	7 ml
4. fT3 Enzyme conjugate: 1 Bottle (ready to use)	7 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 and Equipment Required but 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

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. Specimens 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 specimens.

Reagent Preparation

- Prepare 1X Wash buffer by adding the Wash concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C).
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

1. Format the microwells 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 50 µl of fT3 enzyme conjugate to all wells.
4. Add 50 µl of Anti-T3 Biotin Solution to all the wells.
5. Incubate for 60 minutes at room temperature (18-26°C).
6. 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.
7. Add 100 µl of TMB substrate to all wells.
8. Incubate for 15 minutes at room temperature.
9. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

Calculation of Results

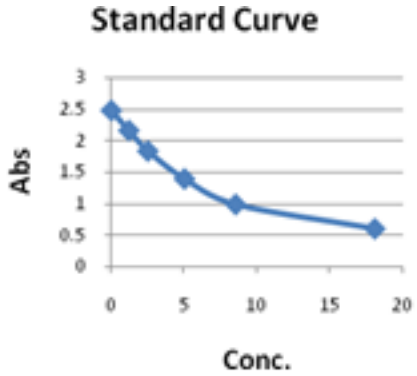
The standard curve is constructed as follows:

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 a Standard Curve

	Conc. ng/ml	OD 450 nm
Std 1	0	2.467
Std 2	1.2	2.149
Std 3	2.5	1.823
Std 4	5.0	1.383
Std 5	8.5	0.976
Std 6	18.0	0.591



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 and may be used as initial guideline ranges only:

Adult 1.4-4.2 pg/ml

Limitation of the Test
The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patients' history, physical findings and other diagnostic procedures.

References

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Version 3, last updated October 18, 2015