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Product Information

Free Thyroxine (fT4) ELISA kit

Catalog Number: EA100836
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The fT4 ELISA kit is used for the quantitative measurement of free Thyroxine (fT4) in human serum.

Background

Over 99% of thyroxine (T4) circulates in blood is bound to carrier proteins; thyroxine-binding globulin (TBG). However, only the free (unbound) portion of Thyroxine 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 T4 level changes so that the free T4 concentration remains constant. Thus, measurements of free T4 concentrations correlate more reliably with clinical status than total T4 levels. The increase in total T4 levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T4 levels while the free T4 concentration remains basically unchanged.

Principle of the test

The fT4 is a solid phase competitive ELISA. The samples Anti-T4 Biotin and fT4 enzyme conjugate are added to the wells coated with Streptavidin. fT4 in the patient's serum competes with a T4 enzyme conjugate for binding sites. Unbound T4 and T4 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 fT4 in the samples. A standard curve is prepared relating color intensity to the concentration of the fT4.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. fT4 Standard: 6 vials (ready to use)	0.5 ml
3. Anti-T4 Biotin Solution	7 ml
4. fT4 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 contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature.

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 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 fT4 standards, control and samples into the assigned well.
3. Add 50 µl of fT4 enzyme conjugate to all wells.
4. Add 50 µl of anti-T4 Biotin Solution to all the wells.
5. Swirl the microplate gently for 20-30 seconds to mix the reagents.
6. Incubate for 60 minutes at room temperature (18-26°C).
7. 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.
8. Add 100 µl of TMB substrate to all wells.
9. Incubate for 15 minutes at room temperature.
10. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
11. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

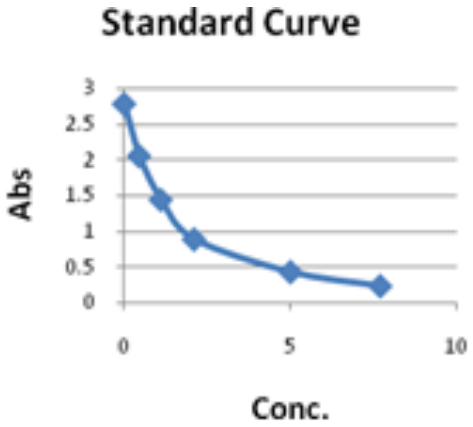
Calculation of Results

The standard curve is constructed as follows:

1. Check fT4 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 fT4 standards (vertical axis) versus fT4 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/dL	OD450 nm
Std 1	0	2.786
Std 2	0.45	2.056
Std 3	1.10	1.440
Std 4	2.10	0.885
Std 5	5.00	0.426
Std 6	7.70	0.229



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 fT4 were established and may be used as initial guideline ranges only:

Adult: 0.8-2.0 ng/dl

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.

References

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