

CALDON BIOTECH INC.
Free Thyroxine (FT4)
ELISA

Catalog No. FT4074
 (96 tests)

INTENDED USE

The CBI FT4 ELISA kit is used for the quantitative measurement of free Thyroxine (FT4) in human serum or plasma.

CLINICAL UTILITY

Over 99% of thyroxine (T4) circulates in blood is bound to carrier proteins; thyroxine-binding globulin (TBG). However, only the free (unbound) portion of triiodothyronine 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 CBI FT4 is a solid phases competitive ELISA. The samples, assay buffer and T4 enzyme conjugate are added to the wells coated with anti-T4 monoclonal antibody. FT4 in the patient's serum competes with a T4 enzyme (HRP) conjugate for binding sites. Unbound FT4 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 FT4.

MATERIALS PROVIDED

1. Microwell strips coated with T4 MAb (12x8x1 wells). Total of 96 wells.
2. FT4 Standard: 6 vials (0.7 mL each). Ready to use.
3. Conjugate Diluent : One bottle 12 mL. Ready to use.
4. TMB Substrate: 1 bottle (12 mL). Ready to use.
5. Stop Solution: 1 bottle (8 mL). Ready to use.
6. T4 Enzyme (HRP) Conjugate Concentrate: 1 vial (20X, 0.7 mL).

7. 10X Wash Concentrate: 1 bottle (50 mL).

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.

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, 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. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens 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.

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.

REAGENTS PREPARATION

1. T4 Enzyme Conjugate Concentrate: Prepare working dilution at 1:20 with conjugate diluent as needed, e.g. 0.1 mL of the stock conjugate in 1.9 mL of conjugate diluent is sufficient for 20 wells. The diluted conjugate has to be used the same day.
2. 10X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 450 ml of distilled water. Store at room temperature.

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 50 μ L of FT4 standards, control and samples.
3. Add 100 μ L of the proper working T4-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 μ L working wash buffer. Wash three times. Blot on absorbent paper towels.
6. Add 100 μ L of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 μ L of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 20 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. See example of the standard attached.
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.

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 by the CBI and may be used as initial guideline ranges only:

Classification	ng/dL
Adult	0.8-2.0

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.

PERFORMANCE CHARACTERISTICS**1. Correlation with a Reference ELISA kit:**

A total of 140 sera were tested by CBI FT4 ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.94	0.90	0.22

2. Precision**Intra-Assay**

Serum	No. of Replicates	Mean ng/dL	Standard Deviation	Coefficient of Variation%
Normal	16	0.89	0.1	11.2
Low	16	1.6	0.14	8.7
High	16	2.3	0.18	7.8

Inter assay

Serum	No. of Replicates	Mean ng/dL	Standard Deviation	Coefficient of Variation%
Normal	10	0.95	0.12	12.6
Low	10	1.4	0.13	9.3
High	10	2.6	0.21	8.0

3. 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 ng/dL	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.41	0.04	0.49 ng/dL

4. Recovery

Known quantities of FT4 were added to a serum that contained a low concentration of FT4.

Expected Value (ng/dL)	Recovered (ng/dL)	Percentage of Recovery
1.2	1	83
1.9	2.2	115
3.1	2.9	94

5. Linearity

Three different patient samples were diluted with the "0" calibrator to 1:2, 1:4 and 1:8. The FT4 values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Serum	Original Value (pg/dL)	Percentage of Recovery		
		1:2	1:4	1:8
1	1.2	101	96	114
2	1.9	95	108	88
3	3.1	89	98	104

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