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**Warning**

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# Free Thyroxine (ft4) ELISA

Catalog No. BQ107T (96 tests)

**INTENDED USE**

The ft4 ELISA kit is used for the quantitative measurement of free Thyroxine (ft4) in human serum or plasma.

**SUMMARY AND EXPLANATION**

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, 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		48 tests	96 tests
1.	Microwell coated with ft4 MAb	6x8x1	12x8x1
2.	ft4 Standard: 6 vials ( ready to use)	0.35ml	0.7ml
3.	ft4 enzyme conjugate: 1 Bottle (ready to use)	6 ml	12 ml
4.	TMB Substrate: 1 bottle (ready to use)	12ml	12ml
5.	Stop Solution: 1 bottle (ready to use)	12ml	12ml
6.	20X Wash concentrate: 1 bottle	25ml	25ml

**MATERIALS NOT PROVIDED**

1. Distilled or de ionized 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 Microwell sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

**WARNINGS AND PRECAUTIONS**

- 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.
- Not for internal or external use in humans or animals
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- 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.
- 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**

- Collect blood specimens and separate the serum immediately.
- 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.
- Avoid multiple freeze-thaw cycles.
- Prior to assay, frozen sera should be completely thawed and mixed well.
- 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(18-26°C).

**ASSAY PROCEDURE**

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C).

- Format the microplates' wells for control, standards and patient sample to be assayed in duplicate.  
Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
- Pipette 50 µl of FT4 standards, control and samples into the assigned well.
- Add 100 µl of FT4 enzyme conjugate to all wells.
- Incubate for 60 minutes at room temperature (18-26° C).
- 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.
- Add 100 µl of TMB substrate to all wells.
- Incubate for 15 minutes at room temperature.
- Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
- 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:
- 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.
- 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.
- 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 and may be used as initial guideline ranges only:

Classification	ng/dL
Adult	0.8-2.0

**LIMITATIONS 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 patient's history, physical findings and other diagnostic procedures.
- 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 FT4 ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.94	0.93	0.09

**2. Precision**

**Intra-Assay**

Serum	No. of Replicates	Mean ng/dL	Standard Deviation	Coefficient of Variation (%)
1	20	0.55	0.061	10.98
2	20	1.74	0.074	4.26
3	20	3.25	0.106	3.25

**Inter Assay**

Serum	No. of Replicates	Mean ng/dL	Standard Deviation	Coefficient of Variation (%)
1	10	0.48	0.052	10.81
2	10	1.41	0.085	6.01
3	10	3.49	0.079	7.90

**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	Mean + 2SD (Sensitivity)
Zero Standard	0.05 ng/dL

**4. Specificity**

The cross reactivity of the thyroxine antibody, used for free T4 EIA to selected substances was evaluated by adding massive amounts the interfering substance to a serum matrix. The cross reactivity was calculated by deriving a ratio between doses of interfering substance to dose thyroxine needed to displace the same amount of the conjugate.

Substance	Cross Reactivity	Concentration
l-Thyroxine	1.0000	-
d-Thyroxine	0.9800	10 µg/dl
d-triiodothyronine	0.0150	100 µg/dl
l-triiodothyronine	0.0300	100 µg/dl
Iodothyrosine	0.0001	100 µg/ml
Diiodothyrosine	0.0001	100 µg/ml
Diiodothyronine	0.0001	100 µg/ml
TBG	N/D	40 µg/ml
Albumin	N/D	40 µg/ml
Phenylbutazone	N/D	10 µg/ml
phenytoin	N/D	40 µg/ml
Salicylates	N/D	500 µg/ml