REFERENCES

1. Baker, S.B., “determination of protein bound Iodine”, Jornal Biological Chemistry 173, 175. (1948)
2. Chopra, I.J., Solomon, D.H., and Ho, R.S., “ A Radioimmunoassay of Thyroxine”, Jornal EndocrainoL 33, 865. (1971)
3. Young, D.S., Pestanger, L.C, and Giberman, U., “Effect ofDrugs on Clinical Laboratory Tests” , clinical chemistry 21, 3660.(1975)
4. Sterling, L., Diagnosis and Treatment of thyroid disease, Cleveland, CRC press P.19-51.(1975)
5. Halpem, E.P. and Bordens RW. “Microencapsulated antibodies in radioimmunoassay. Determination of free thyroxine”
6. Stjernholm, MR, Alsever, RN and Rudolph, MC. “Thyroid function test in diphenylhydantoin-treated patients”, clin. Chem.. vol. 21, 1388-1392. (1977)
7. Nelson, J.C. and Wilcox, RB. “ Analytical performance of free and total thyroxine assays”. Clin. Chem. Vol. 42, 146-154 (1996)
8. Midgeley john , EM. “ Direct and Indirect free thyroxine assay methods. Theory and practice” clin. Chem. Vol. 47, 1353-1363. (2001)
9. Bayer, MF and McDougall, IR. “ Radioimmunoassay of free thyroxine in serum. Comparison with clinical finding and results of conventional thyroid-function tests”clin. Chem. Vol. 26, 1186-1192. (1980)
10. Anthony, GW. Jackson, RA et. al. “Misleading results from immunoassays of serum free thyroxine in the presence of rheumatoid factor”, Clin. Chem vol. 43, 957-962. (1997)
11. Wosillait, WD. “ A theoretical analysis of the distributionof thyroxine among site on the thyroxine binding globulin, thyroid binding prealbumiun and serum albumin “. RES. Comm. Chem. Patho-pharmacology 16, 541-548. (1977).

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Manufactured For:
Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave NE, Suite P
Minneapolis, MN 55432
Tel: (763) 780-2955 / Fax: (763) 780-2988
www.IBL-America.com
info@ibl-america.com

Free Thyroxine (fT4) ELISA

Catalog No. IB19106 (96 Tests)

INTENDED USE

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

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 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.

MATERIALS PROVIDED		96 Tests
1.	Microwells coated with Streptavidin	12x8x1
2.	fT4 Standard: 6 vials (ready to use)	0.5 ml
3.	fT4 Control: 2 vials (ready to use)	0.5 ml
4.	Anti-T4 Biotin Solution	7 ml
5.	fT4 Enzyme conjugate: 1 Bottle (ready to use)	7 ml
6.	TMB Substrate: 1 bottle (ready to use)	12 ml
7.	Stop Solution: 1 bottle (ready to use)	12 ml
8.	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 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.

SAMPLE COLLECTION & HANDLING

1. Collect blood samples 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 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 (20-25 °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 25 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.

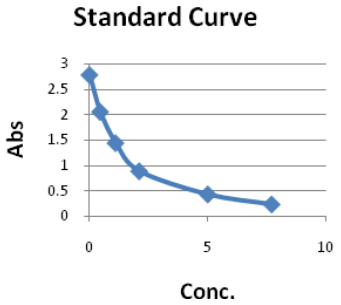
CALCULATION OF RESULTS

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 STANDARD CURVE

	OD 450 nm	Conc. ng/dL
Std 1	2.875	0
Std 2	2.011	0.45
Std 3	1.378	1.10
Std 4	0.929	2.10
Std 5	0.398	5.00
Std 6	0.198	7.70



EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges.

LIMITATIONS OF THE TEST

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