LIMITATIONS OF THE TEST

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

REFERENCES

1. Agharanya JC. Clinical usefulness of ELISA technique in the assessment of thyroid function. West Afr J Med 1990;9(4):258-63.
2. Frank JE; Faix JE; Hermos RJ; Mullaney DM; Rojan DA; Mitchell ML; Klein RZ Thyroid function in very low birth weight infants: effects on neonatal hypothyroidism screening. J Pediatr 1996;128(4):548-54.
3. Shimada T; Higashi K; Umeda T; Sato T. Thyroid functions in patients with various chronic liver diseases. Endocrinol Jpn 1988;35(3):357-69.
4. Thakur C; Saikia TC; Yadav RN. Total serum levels of triiodothyronine (T3) thyroxine (T4) and thyrotropine (TSH) in school going children of Dibrugarh district: an endemic goitre region of Assam. Indian J Physiol Pharmacol 1997;41(2):167-70.

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Thyroxine (T4) ELISA

Catalog No. IB19108 (96 tests)

INTENDED USE

The Thyroxine (T4) ELISA Kit is intended for the detection of Total T4 in human serum or plasma. For research use only. Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

T4 is a useful marker in the studies of hypothyroidism and hyperthyroidism. The level of T4 is decreased in hypothyroid samples and is increased in hyperthyroid samples. The level of T4 is normal in Euthyroid samples.

PRINCIPLE OF THE TEST

The T4 is a solid phases competitive ELISA. The samples, working T4-HRP Conjugate and Anti-T4-Biotin Solution are added to the wells coated with Streptavidin. T4 in the sample competes with a T4 enzyme (HRP) 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 T4 in the samples. A standard curve is prepared relating color intensity to the concentration of the T4.

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. T4 Standard: 6 vials (ready to use)	0.5ml
3. T4 Control: 2 vials (ready to use)	0.5ml
4. Anti-T4-Biotin Solution (ready to use)	7ml
5. T4-HRP Conjugate (11X)	0.7ml
6. T4 Assay Diluent	7ml
7. TMB Substrate: 1 bottle (ready to use)	12ml
8. Stop Solution: 1 bottle (ready to use)	12ml
9. 20X Wash concentrate: 1 bottle	25ml

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, 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. For Research Use Only. Not for use in Diagnostic Procedures.
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.

REAGENT PREPARATION

1. **T4-enzyme Conjugate Solution**
   Dilute the T4-enzyme conjugate 1:11 with Total conjugate buffer in a suitable container. For example, dilute 80µl of enzyme conjugate with 0.8ml of assay diluent for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C.

   General Formula:
   Amount of Buffer required = Number of wells * 0.05
   Quantity of Enzyme conjugate solution necessary = # of wells * 0.005
   i.e. = 16 x 0.05 = 0.8ml for Total Conjugate Buffer
   16 x 0.0005 = 0.08ml (80µl) for enzyme conjugate solution.

2. **Wash Buffer**
   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).

1. Format the microplates' wells for each serum reference, control and unknown specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 25µl of the standards, control or sample into the assigned well.
3. Add 50µl of the working T4-enzyme conjugate solution to all wells (see Reagent Preparation Section).
4. Add 50µl of T4-Antibody-Biotin Solution to all wells.
5. Swirl the microplate gently for 20-30 seconds to mix the reagents.
6. Cover and Incubate 60 minutes at room temperature.
7. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
8. Add 100µl of TMB substrate solution to all wells
9. Cover the plate and incubate at room temperature for fifteen (15) minutes.
10. Add 50µl of stop solution to each well and gently mix for 15-20 seconds.
11. Read the absorbance on ELISA Reader for each well at 450nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check T4 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 T4 standards (vertical axis) versus T4 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

	OD 450 nm	Conc. µg/dL
Std 1	2.18	0
Std 2	1.50	2
Std 3	1.19	5
Std 4	0.86	10
Std 5	0.65	15
Std 6	0.44	25