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. Baumgartner-Parzer SM; Wagner L; Reining G; Sexl V; Nowotny P; Muller M; Brunner M; Waldh¨ausl W. Increase by tri-iodothyronine of endothelin-1, fibronectin and von Willebrand factor in cultured endothelial cells. J Endocrinol 1997;154(2):231-9.
3. Maes M; Mommen K; Hendrickx D; Peeters D; D'Hondt P; Ranjan R; De Meyer F; Scharp´e S Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clin Endocrinol (Oxf) 1997; 46(5):587-98.
4. Santini F; Chiovato L; Bartalena L; Lapi P; Palla R; Panichi V; Velluzzi F; Grasso L; Chopra IJ; Martino E; Pinchera A Study of serum 3,5,3'-triiodothyronine sulfate concentration in patients with systemic non-thyroidal illness. Eur J Endocrinol 1996;134(1):45-9

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Cat#: T3043T-100 (96 tests)
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CALBIOTECH
A life science company

Mouse/Rat Triiodothyronine (T3) ELISA

Catalog No. T3043T-100 (96 tests)

INTENDED USE

For Research Use Only. Not for use in diagnostic procedures.

MATERIALS PROVIDED	96 tests
1. Microwells coated with T3 Monoclonal Ab	12x8x1
2. T3 Standard: 7 vials (ready to use)	0.25ml
3. T3 Enzyme Conjugate concentrate: 1 vial	1.5ml
4. Assay diluent: (ready to use)	12 ml
5. TMB Substrate: 1 bottle (ready to use)	12ml
6. Stop Solution: 1 bottle (ready to use)	12ml
7. 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. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials:
   The calibrator and controls contain animal and 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.

4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. 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. Typically, 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. **T3-enzyme Conjugate Solution**
   Dilute the T3-enzyme conjugate 1:11 with assay diluent in a suitable container. For example, dilute 160µl of conjugate with 1.6ml 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 * 0.1
   Quantity of T3-Enzyme necessary = # of wells * 0.01
   i.e. = 16 x 0.1 = 1.6ml for Assay diluent
   16 x 0.01 = 0.16ml (160µl) for T3 enzyme conjugate
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 (20-25°C).

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C).

1. Format the microplate wells for each serum reference, control and patient 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 appropriate serum reference, control or specimen into the assigned well.
3. Add 100µl of workingT3-enzyme conjugate solution to all wells (see Reagent Preparation Section).
4. Cover the plate and Incubate for 60 minutes at room temperature with shaking at 650rpm.
5. Remove liquid from all wells. Wash wells three times with 300 of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
6. Add 100µl of TMB substrate solution to all wells
7. Cover the plate and Incubate at room temperature for fifteen (15) minutes.
8. Add 50µl of stop solution to each well and gently mix for 15-20 seconds.
9. Read the absorbance on ELISA Reader of each well at 450nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check T3 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 T3 standards (vertical axis) versus T3 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. ng/mL
Std 1	2.404	0
Std 2	2.238	0.25
Std 3	2.001	0.5
Std 4	1.714	1
Std 5	1.147	2.5
Std 6	0.793	5
Std 7	0.642	7.5

LIMITATIONS OF THE TEST

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