REFERENCES
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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.

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For Order and Inquiries, please contact
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Mouse/Rat Total Triiodothyronine (T3) ELISA

Catalog No. T3104T-100 (96 tests)

INTENDED USE

The CALBIOTECH INC. (CBI) T3 ELISA kit is used for the quantitative measurement of total Triiodothyronine (T3) in mouse/rat serum or plasma.

SUMMARY AND EXPLANATION

Triiodothyronine (T3) is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T3 is decreased in hypothyroid and is increased in hyperthyroid , graves disease and pregnancy.

PRINCIPLE OF THE TEST

The CBI T3 is a solid phase competitive ELISA. The samples, assay buffer and T3 enzyme conjugate are added to the wells coated with anti-T3 monoclonal antibody. T3 in serum competes with a T3 enzyme conjugate for binding sites. Unbound T3 and T3 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 T3 in the samples. A standard curve is prepared relating color intensity to the concentration of the T3.

MATERIALS PROVIDED	96 tests
1. Microwell coated with T3 MAb	12x8x1
2. T3 Standard: 6 vials (ready to use)	0.7ml
3. Conjugate Buffer	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12ml
5. Stop Solution: 1 bottle (ready to use)	12ml
6. T3 Enzyme Conjugate concentrate 1 vial	1.5ml
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.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain animal 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 test 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.

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. T3-enzyme Conjugate Solution:
   Dilute the T3-enzyme conjugate 1:11 with Total T3 conjugate buffer in a suitable container.
   For example, dilute 160µl of conjugate with 1.6ml of buffer 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 Total T3 Conjugate Buffer
   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 (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 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 50µl of the appropriate serum reference, control or specimen into the assigned well.
3. Add 100µl of T3-enzyme conjugate solution to all wells (see Reagent Preparation Section).
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
7. Add 100µl of TMB substrate solution to all wells.
8. Incubate at room temperature for fifteen (15) minutes.
9. Add 50µl of stop solution to each well and gently mix for 15-20 seconds.
10. 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.
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.

LIMITATIONS OF THE TEST

1. The test results obtained using this kit is research use. It's recommended that each lab establish a normal range based on sample population.
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 120 sera were tested by CBI T3 ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.98	0.94	3.8

2. Precision
   a. Intra-Assay Precision was determined by assaying 16 replicates of each of three sera; low, normal, and high.

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	16	0.78	0.06	7.4
2	16	1.92	0.10	5.4
3	16	3.55	0.14	3.9

   b. Inter assay Precision was determined by assaying duplicates of three serum pools in 10 separate runs, using a standard curve constructed for each run.

Serum	No. of Runs	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	10	0.76	0.07	8.9
2	10	1.85	0.13	6.7
3	10	3.43	0.16	4.5

3. Sensitivity

The Triiodothyronine (T3) test system procedure has a sensitivity of 0.04 ng/ml. the sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2da(95% certainty) statistics to calculate the minimum dose.