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Mouse/Rat Thyroxine (T4) ELISA

Catalog No. T4044T-100 (96 Tests)

INTENDED USE

The Calbiotech Mouse/Rat T4 ELISA Kit is intended for the detection of total T4 in mouse or rat serum or plasma. This procedure provides ultra sensitivity and minimum sample volume (10ul per sample) which are very critical for testing Mouse or Rat samples. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Thyroxine (T4) is the primary circulating thyroid hormone, converted into T3 in target tissues. This assay detects T4 in rodent samples for research on thyroid hormone biosynthesis, transport, and systemic metabolic effects.

MATERIALS PROVIDED	96 TESTS
1. Microwells coated with T4 Monoclonal Ab	12x8x1
2. T4 Standard: 7 vials (ready to use)	0.25 mL
3. T4 Control: 2 vials (ready to use)	0.25 mL
4. T4 Enzyme (HRP) Conjugate concentrate: 1 vial	1.5 mL
5. Assay Diluent (ready to use)	12 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

Potential biohazardous materials:

1. The standards 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. 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

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

REAGENT PREPARATION

1. **T4-enzyme Conjugate Solution**
   Dilute the T4-enzyme conjugate 1:11 with assay diluent in a suitable container. For example, dilute 160µl of enzyme 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 Enzyme conjugate solution necessary = # of wells * 0.01
   i.e. = 16 x 0.1 = 1.6ml for Total Conjugate Buffer
   16 x 0.01 = 0.16ml (160µl) for enzyme conjugate solution.

ASSAY PROCEDURE

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

1. Format the microplates' 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 10µl of the standards, control or specimen into the assigned well.
3. Add 100µl of T4-enzyme conjugate solution to all wells (see Reagent Preparation Section).
4. Incubate for 60 minutes at room temperature with shaking.
5. 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.
6. Add 100µl of TMB substrate solution to all wells
7. Incubate, at room temperature, for fifteen (15) minutes.
8. Add 50µl of stop solution to all wells and gently mix for 15-20 seconds.
9. 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	2.615	0
Std 2	1.982	1
Std 3	1.627	2
Std 4	1.075	5
Std 5	0.646	10
Std 6	0.471	15
Std 7	0.325	25

LIMITATIONS OF THE TEST

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