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Triiodothyronine (T3) ELISA Kit

Name	Triiodothyronine (T3) ELISA Kit
INTENDED USE	The Triiodothyronine (T3) ELISA Kit is intended for the quantitative measurement of Triiodothyronine (T3) in human serum.
SUMMARY AND EXPLANATION	Triiodothyronine (T3) is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T3 is decreased in hypothyroid patients and is increased in hyperthyroid patients, graves disease and pregnancy.
PRINCIPLE OF THE TEST	The T3 is a solid phases competitive ELISA. The samples, assay buffer and T3 enzyme conjugate are added to the wells coated with anti-T3 monoclonal antibody. T3 in the patient's 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
Microwell coated with T3 MAb	12x8x1
T3 Standard: 6 vials (ready to use)	0.5 ml
T3 Assay Diluent	12 ml
TMB Substrate: 1 bottle (ready to use)	12 ml
Stop Solution: 1 bottle (ready to use)	12 ml
T3 Enzyme Conjugate concentrate, 11X: 1 vial	1.2 ml
20X concentrate: 1 bottle	25 ml

MATERIALS NOT PROVIDED	Distilled or deionized water

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	Precision pipettes
	Disposable pipette tips
	ELISA reader capable of reading absorbance at 450nm
	Absorbance paper or paper towel
	Graph paper
STORAGE AND STABILITY	1. Store the kit at 2 – 8 C.
	Keep microwells sealed in a dry bag with desiccants.
	The reagents are stable until expiration of the kit.
	Do not expose test reagents to heat, sun, or strong light.
WARNINGS AND PRECAUTIONS	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.
	This test kit USA FDA exempt product.
	Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
	The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
	It is recommended that standards, control and serum samples be run in duplicate.
	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	Collect blood specimens and separate the serum immediately.
	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. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens.
REAGENT PREPARATION	T3-Enzyme Conjugate Solution Dilute the T3-enzyme conjugate 1:11 with 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 Conjugate Buffer 16 x 0.01 = 0.16ml (160µl) for T3 enzyme conjugate 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). 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. Pipette 50µl of the appropriate serum reference, control or specimen into the assigned well. Add 100µl of T3-enzyme conjugate solution to all wells (see Reagent Preparation Section). Swirl the microplate gently for 20-30 seconds to mix and cover. Incubate 60 minutes at room temperature. Remove liquid from all wells. wells three times with 300 of

1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.

Add 100µl of TMB substrate solution to all wells

Incubate at room temperature for fifteen (15) minutes.

Add 50µl of stop solution to each well and gently mix for 15-20 seconds.

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:

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.

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.

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	2.568	0
Std 2	1.706	0.75
Std 3	1.223	1.5
Std 4	0.611	3.5
Std 5	0.330	6
Std 6	0.186	9

LIMITATIONS OF THE TEST

The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patients history, physical findings and other diagnostic procedures.

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

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