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Thyroxine (T4) ELISA Kit

Name	Thyroxine (T4) ELISA Kit
INTENDED USE	The Thyroxine (T4) ELISA Kit is intended for the detection of Total T4 in human serum or plasma.
SUMMARY AND EXPLANATION	T4 is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T4 is decreased in hypothyroid patients and is increased in hyperthyroid patients. The level of T4 is normal in Euthyroid individuals.
PRINCIPLE OF THE TEST	The T4 kit is a solid phase competitive ELISA. The samples, working T4-HRP Conjugate and Anti-T4-Biotin Solution are added to the wells coated with Streptavidin. T4 in the patient's serum 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
Microwells coated with Streptavidin	12x8x1
T4 Standard: 6 vials (ready to use)	0.5ml
Anti-T4-Biotin Solution (ready to use)	7ml
T4-HRP Conjugate 11X	0.7ml
T4 Assay Diluent	7ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
20X Wash concentrate: 1 bottle	25ml

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MATERIALS NOT PROVIDED	Distilled or deionized water
	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, 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
	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

1. T4-enzyme Conjugate Solution

Dilute the T4-enzyme conjugate 1:11 with assay diluent 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 0.005 = 0.08ml (80µl) for enzyme conjugate solution.

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 25µl of the standards, control or specimen into the assigned well.

Add 50µl of the working T4-enzyme conjugate solution to all wells (see Reagent Preparation Section).

Add 50µl of T4-Antibody-Biotin Solution to all wells.

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Swirl the microplate gently for 20-30 seconds to mix the reagents.

Cover and Incubate 60 minutes at room temperature.

Remove liquid from all wells. wells three times with 300 µl of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.

Add 100µl of TMB substrate solution to all wells

Cover the plate and 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 for each well at 450nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

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.

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.

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

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.

EXPECTED VALUES

A study of euthyroid in adult population was undertaken to determine expected values for the T4 EIA test system. The mean value, standard deviation and expected ranges of samples are presented in the following table:

	Male (42 samples)	Female (58 samples)
Mean	7.6	8.2
Standard deviation	1.6	1.7
Expected range	4.4-10.8	4.8-11.6

REFERENCES

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