

CALDON BIOTECH INC. Thyroxine (T4) ELISA

Catalog No. T4044T
(96 tests)

INTENDED USE

The CBI T4 ELISA kit is used for the quantitative measurement of total Thyroxine (T4) in human serum or plasma.

CLINICAL UTILITY

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 CBI T4 is a solid phases competitive ELISA. The samples, assay buffer and T4 enzyme conjugate are added to the wells coated with anti-T4 monoclonal antibody. 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

1. Microwell strips coated with T4 MAb (12x8x1 wells). Total of 96 wells.
2. T4 Standard: 6 vials (0.7 mL each). Ready to use.
3. Conjugate Diluent: 12 mL. Ready to use.
4. TMB Substrate: 1 bottle (12 mL). Ready to use.
5. Stop Solution: 1 bottle (8 mL). Ready to use.
6. T4 Enzyme (HRP) Conjugate Concentrate: 1 vial (20X, 0.7 mL).
7. 10X Wash Concentrate: 1 bottle (50 mL).

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 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. This test kit is designed for in vitro diagnostic 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.

REAGENTS PREPARATION

1. T4 Enzyme Conjugate Concentrate: Prepare working dilution at 1:20 with conjugate diluent as needed, e.g. 0.1 mL of the stock conjugate in 1.9 mL of conjugate diluent is sufficient for 20 wells. The diluted conjugate has to be used the same day.
2. 10X Wash Buffer Concentrate: To prepare working wash buffer, add the

contents of the bottle to 450 ml of distilled water. Store at room temperature.

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature.

Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 25 µL of T4 standards, control and patient's sera.
3. Add 100 µL of working T4-enzyme Conjugate to all wells.
4. Incubate for 60 minutes at room temperature (18-26° C).
5. Remove liquid from all wells. Fill wells with working wash buffer. Wash three times. Blot on absorbent paper towels.
6. Add 100 µL of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µL of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 20 minutes after adding the stopping 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.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for T4 were established by the CBI and may be used as initial guideline ranges only:

Classification	µg/dL
Normal Males	5.3-10.5
Normal Females	5.7-11.4
1-11 Months	7.2-15.7
Newborn (1-4 days)	14-28

LIMITATIONS OF THE TEST

1. 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.
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 140 sera were tested by CBI T4 ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.97	0.91	0.27

2. Precision

Intra-Assay

Serum	No. of Replicates	Mean µg/dL	Standard Deviation	Coefficient of Variation%
Normal	16	6.83	0.74	10.8
Low	16	3.20	0.31	9.7
High	16	18.80	2.38	12.6

Inter assay

Serum	No. of Replicates	Mean µg/dL	Standard Deviation	Coefficient of Variation%
Normal	10	8.40	0.848	9.9
Low	10	3.56	0.40	11.5
High	10	16.10	2.0	12.4

3. Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean µg/d	Standard deviation	Mean + 2SD (Sensitivity)

		L	Deviation	y)
Zero Standard	20	0.74	0.38	1.5 μ g/dL

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1997;41(2):167-70.

4. Recovery

Known quantities of T4 were added to a serum that contained a low concentration of T4.

Expected Value (μ g/dL)	Recovered (μ g/dL)	Percentage of Recovery
2.80	2.76	98.6
10.0	11.25	112.5
10.0	10.63	106.3

5. Linearity

Three different patient samples were diluted with the "0" calibrator to 1:2, 1:4 and 1:8. The T4 values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Serum	Original Value (μ g/dL)	Percentage of Recovery		
		1:2	1:4	1:8
1	26	103.4	106.2	114.7
2	30	89.3	118.0	118.0
3	21	85.7	87.6	114.1

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