

LIMITATIONS OF THE TEST

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

REFERENCES

1. Agharanya JC. Clinical usefulness of ELISA technique in the assessment of thyroid function. West Afr J Med 1990;9(4):258-63.
2. Frank JE; Faix JE; Hermos RJ; Mullaney DM; Rojan DA; Mitchell ML; Klein RZ Thyroid function in very low birth weight infants: effects on neonatal hypothyroidism screening. J Pediatr 1996;128(4):548-54.
3. Shimada T; Higashi K; Umeda T; Sato T. Thyroid functions in patients with various chronic liver diseases. Endocrinol Jpn 1988;35(3):357-69.
4. Thakur C; Saikia TC; Yadav RN. Total serum levels of triiodothyronine (T3) thyroxine (T4) and thyrotropine (TSH) in school going children of Dibrugarh district: an endemic goitre region of Assam. Indian J Physiol Pharmacol 1997;41(2):16 7-70.

2018-06-01

Cat#: IB19107N (96 Tests)

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Triiodothyronine (T3) ELISA

Catalog No. IB19107N (96 Tests)

INTENDED USE

The Triiodothyronine (T3) ELISA Kit is intended for the quantitative measurement of Triiodothyronine (T3) in human serum. For research use only – not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

Triiodothyronine (T3) is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T3 is decreased in hypothyroid samples and is increased in hyperthyroid samples, 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 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)	0.5ml
3. T3 Assay Diluent	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12 ml
5. Stop Solution: 1 bottle (ready to use)	12 ml
6. T3 Enzyme Conjugate concentrate, 11X: 1 vial	1.2 ml
7. 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

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 Research Use Only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which samples 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.

SAMPLE COLLECTION HANDLING

1. Collect blood samples and separate the serum immediately.
2. Samples 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 samples.

REAGENT PREPARATION

1. **T3-enzyme Conjugate Solution**
Dilute the T3-HRP conjugate 1:11 with assay diluent in a suitable container. For example, for an entire plate, dilute 1.2 mL T3 enzyme conjugate with 12mL of assay diluent. (A slight excess of solution is made). **Prepare only what you need for the number of strips you are running that day.** This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C.
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 sample 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 standards, control or sample into the assigned well.
3. Add 100µl of the T3-enzyme conjugate solution to all wells (see Reagent Preparation Section).
4. Swirl the microplate gently for 20-30 seconds to mix the reagents.
5. Cover and Incubate 60 minutes at room temperature.
6. 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.
7. Add 100µl of TMB substrate solution to all wells
8. Cover the plate and 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 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 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.
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.

Refer to Certificate Analysis for Lot specific standard curve example.