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Product Information

Triiodothyronine (T3) ELISA kit

Catalog Number: EA100838
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Mouse/Rat Triiodothyronine (T3) ELISA Kit is intended for the quantitative measurement of Triiodothyronine (T3) in mouse/rat serum or plasma.

Background

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. The level of T3 is normal in euthyroid individuals.

Principle of the test

The OriGene Mouse/Rat T3 is a solid phase competitive ELISA. The samples, the working T3 enzyme conjugate, diluted in assay diluent, are added to the wells coated with anti-T3 polyclonal antibody. T3 in the patient's serum competes with 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.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with T3 polyclonal antibody	12x8x1
2. T3 Standard: 7 vials (ready to use)	0.25 ml
3. Assay Diluent: 1 bottle	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: 1 vial	1.5 ml
7. 20X Wash concentrate: 1 bottle	25 ml

Materials and Equipment Required but 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

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazards materials:
   The calibrator and controls contain animal and 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.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. 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. Typically, specimens may be stored refrigerated at (2-8 ^{o}C) for 5 days. If storage time exceeds 5 days, store frozen at (-20 ^{o}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

Reagent Preparation

1. T3-enzyme Conjugate Solution

Dilute the T3-enzyme conjugate 1:11 with assay diluent in a suitable container. For example, dilute 160µl of conjugate with 1.6ml 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 * 0.1
Quantity of T3-Enzyme necessary = # of wells * 0.01
i.e. = 16 x 0.1 = 1.6ml for Assay diluent
16 x 0.01 = 0.16ml (160µl) for T3 enzyme conjugate

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 (20-25°C).

Assay Procedure

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

1. Format the microplate 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 25µl of the appropriate serum reference, control or specimen into the assigned well.
3. Add 100µl of workingT3-enzyme conjugate solution to all wells (see Reagent Preparation Section).
4. Cover the plate and Incubate for 60 minutes at room temperature with shaking at 650rpm.
5. Remove liquid from all wells. Wash wells three times with 300 of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
6. Add 100µl of TMB substrate solution to all wells
7. Cover the plate and Incubate at room temperature for fifteen (15) minutes.
8. Add 50µl of stop solution to each well and gently mix for 15-20 seconds.
9. 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:

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.

Example of a Standard Curve

	Conc. ng/ml	OD 450 nm
Std 1	0	2.404
Std 2	0.25	2.238
Std 3	0.5	2.001
Std 4	1	1.714
Std 5	2.5	1.147
Std 6	5	0.793
Std 7	7.5	0.642

Limitation of the Test

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.

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3. Maes M; Mommen K; Hendrickx D; Peeters D; D'Hondt P; Ranjan R; De Meyer F; Scharp´e S Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clin Endocrinol (Oxf) 1997; 46(5):587-98.

4. Santini F; Chiovato L; Bartalena L; Lapi P; Palla R; Panichi V; Velluzzi F; Grasso L; Chopra IJ; Martino E; Pinchera A Study of serum 3,5,3'-triiodothyronine sulfate concentration in patients with systemic non-thyroidal illness. Eur J Endocrinol 1996;134(1):45-9

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