



T3 (Total) (Mouse/Rat) ELISA Kit

Catalog Number KA0925

96 assays

Version: 02

Intended for research use only

www.abnova.com

Table of Contents

Introduction	3
Intended Use	3
Background	3
Principle of the Assay	3
General Information	4
Materials Supplied	4
Storage Instruction	4
Materials Required but Not Supplied	4
Precautions for Use	4
Assay Protocol	6
Reagent Preparation	6
Assay Procedure	6
Data Analysis.....	8
Calculation of Results.....	8
Performance Characteristics	8
Resources.....	9
References	9
Plate Layout	10

Introduction

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

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 Assay

The 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.

General Information

Materials Supplied

List of component

Component	Amount
Microwell coated with T3 Polyclonal Ab	96 (12 x 8) wells
T3 Standard: 7 vials (ready to use)	0.25 ml
T3 Enzyme Conjugate concentrate: 1 vial	1.5 ml
Assay diluent: (ready to use)	12 ml
TMB Substrate: 1 bottle (ready to use)	12 ml
Stop Solution: 1 bottle (ready to use)	12 ml
20X Wash concentrate: 1 bottle	25 ml

Storage Instruction

- ✓ 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.

Materials Required but Not Supplied

- ✓ Distilled or deionized water
- ✓ Precision pipettes
- ✓ Disposable pipette tips
- ✓ ELISA reader capable of reading absorbance at 450nm
- ✓ Absorbance paper or paper towel
- ✓ Graph paper

Precautions for Use

- ✓ Warnings and Precautions
- Potential biohazardous 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.

- This test kit is designed For Research Use only.
 - 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.
- ✓ Limit of assay
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Assay Protocol

Reagent Preparation

✓ T3-enzyme Conjugate Solution

Dilute the T3-enzyme conjugate 1:11 with assay diluent in a suitable container. For example, dilute 160 μ l of conjugate with 1.6 ml 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 = 1.6 ml for Assay diluent

16 x 0.01 = 0.16 ml (160 μ l) 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 (18-26°C).

Sample Preparation

- ✓ 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.

Assay Procedure

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C).

- ✓ 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.
- ✓ Pipette 25 μ l of the appropriate serum reference, control or specimen into the assigned well.
- ✓ Add 100 μ l of working T3-enzyme conjugate solution to all wells
- ✓ Cover the plate and Incubate for 60 minutes at room temperature with shaking.
- ✓ Remove liquid from all wells. Wash wells three times with 300 of 1X wash buffer. Blot on absorbent paper towels.
- ✓ Add 100 μ l 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 of each well at 450nm within 15 minutes after adding the stop solution.

Data Analysis

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

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

Performance Characteristics

✓ Expected Values

Using the mouse/rat ELISA kit, the normal serum/plasma sample is expected to contain between 0.5 ng/ml and 1.5 ng/ml T3. It is recommended that each laboratory establish their own range of expected values for the population being tested.

✓ Sensitivity

The Triiodothyronine test system procedure has a sensitivity of 0.186 ng/ml.

Resources

References

- ✓ Agharanya JC. Clinical usefulness of ELISA technique in the assessment of thyroid function. *West Afr J Med* 1990; 9(4):258-63.
- ✓ Baumgartner-Parzer SM; Wagner L; Reining G; Sexl V; Nowotny P; Muller M; Brunner M; Waldh"ausl W. Increase by tri-iodothyronine of endothelin-1, fibronectin and von Willebrand factor in cultured endothelial cells. *J Endocrinol* 1997; 154(2):231-9.
- ✓ 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.
- ✓ 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.

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H