

Product Information

Mouse/Rat Triiodothyronine (T₃) ELISA

Catalog Number **SE120091**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

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.

The Mouse/Rat Triiodothyronine (T₃) ELISA is intended for the quantitative measurement of Triiodothyronine (T₃) in mouse/rat serum or plasma. It is a solid phase competitive ELISA. The samples and the working T₃ Enzyme Conjugate diluted in Assay Diluent, are added to the wells coated with anti-T₃ polyclonal antibody. T₃ in the serum competes with T₃ Enzyme Conjugate for binding sites. Unbound T₃ and T₃ Enzyme Conjugate are washed off. 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
Microwell coated with T ₃ Polyclonal Ab	12 x 8 x 1
T ₃ Standard: 7 vials (ready to use)	0.25 mL
T ₃ 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

Reagents 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 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. 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.

Note: Do not use grossly lipemic specimens.

T₃-enzyme Conjugate Solution

Dilute the T₃-Enzyme Conjugate 11-fold with Assay Diluent in a suitable container. For example, dilute 160 µL of Enzyme Conjugate with 1.6 mL of buffer for 16 wells (A slight excess of solution is made).
Amount of Buffer required = Number of wells x 0.1 mL
Quantity of Enzyme Conjugate solution necessary = number of wells x 0.01 mL
16 x 0.1 = 1.6 mL for Total Conjugate Buffer
16 x 0.01 = 0.16 mL (160 µL) for Enzyme Conjugate solution.

Note: This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2–8 °C.

20x Wash Buffer Concentrate

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).

Storage/Stability

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.

Procedure

Notes: 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.

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

1. Format the microplate wells for each serum reference, control, and 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 25 µL of the appropriate serum reference, control, or specimen into the assigned well.
3. Add 100 µL of working T₃-Enzyme Conjugate solution to all wells (see Preparation Instructions).
4. Cover the plate and incubate for 60 minutes at room temperature with shaking.
5. Remove liquid from all wells. Wash wells three times with 300 of 1x Wash buffer (see Preparation Instructions). Blot on absorbent paper towels.
6. Add 100 µL of TMB substrate solution to all wells.
7. Cover the plate and incubate at room temperature for 15 minutes.
8. Add 50 µL of Stop Solution to each well and gently mix for 15–20 seconds.
9. Read the absorbance on ELISA Reader of each well at 450 nm within 15 minutes after adding the stop solution.

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 the value is checked on every kit.
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.

Typical Data for Standard Curve

	OD (450 nm)	Concentration (ng/mL)
Std 1	2.404	0
Std 2	2.238	0.25
Std 3	2.001	0.5
Std 4	1.714	1
Std 5	1.147	2.5
Std 6	0.793	5
Std 7	0.642	7.5

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Expected Values

Using the Mouse/Rat Triiodothyronine (T₃) ELISA kit, the normal serum/plasma sample is expected to contain between 0.5–1.5 ng/mL of T₃. It is recommended that each laboratory establish their own range of expected values for the population being tested.

Note: Do not use sodium azide as preservative. Sodium azide inhibits HRP Enzyme activities.

References

1. Agharanya, J.C., Clinical usefulness of ELISA technique in the assessment of thyroid function. *West Afr. J. Med.*, 1990;9(4):258-63.
2. Baumgartner-Parzer, S.M. et al., Increase by triiodothyronine of endothelin-1, fibronectin and von Willebrand factor in cultured endothelial cells. *J. Endocrinol.*, 1997; 154(2):231-9.
3. Maes, M. et al., 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. et al., 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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