3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Triiodothyronine (T_3) ELISA

Catalog Number **SE120132**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Triiodothyronine (T_3) is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T_3 is decreased in hypothyroid individuals and is increased in hyperthyroid individuals. The level of T_3 is normal in euthyroid individuals.

The Triiodothyronine (T_3) ELISA Kit is intended for the detection of total T_3 in human serum or plasma. It is a solid phase competitive ELISA. The samples, T_3 antibody-biotin solution, and the diluted T_3 enzyme conjugate are added to the wells coated with streptavidin. T_3 in the serum competes with a T_3 enzyme (HRP) conjugate for binding sites. Unbound T_3 and T_3 enzyme conjugate is washed off. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of T_3 in the samples. A standard curve generated relating color intensity to the concentration of the T_3.

Components

Materials Provided	96 Tests
Microwells coated with Streptavidin	12 x 8 x 1
T_3 Standard: 6 vials (ready to use)	0.5 mL
T_3 HRP Conjugate	0.7 mL
Anti-T_3 Biotin Solution	7 mL
Assay Diluent: 1 bottle	7 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.

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

T_3-enzyme Conjugate Solution

Dilute the T_3-enzyme conjugate 11-fold with assay diluent in a suitable container. For example, dilute 80 µL of enzyme conjugate with 0.8 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.

Note:

Amount of Buffer required = Number of wells x 0.5 mL
(16 x 0.5 = 0.8 mL for Total Conjugate Buffer)

Quantity of Enzyme conjugate solution necessary = number of wells x 0.005 mL
16 x 0.05 = 0.08 mL (80 µL) for enzyme conjugate solution.

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.

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

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 50 µL of the standards, control, or specimen into the assigned well.
3. Add 50 µL of the working T_3-enzyme conjugate solution to all wells (see Preparation Instructions).
4. Add 50 µL of T_3-Antibody-Biotin Solution to all wells.
5. Swirl the microplate gently for 20–30 seconds to mix the reagents.
6. Cover and incubate 60 minutes at room temperature.
7. Remove liquid from all wells. Wash wells three times with 300 µL of 1x wash buffer (see Preparation Instructions). Blot on absorbent paper towels.
8. Add 100 µL of TMB substrate solution to all wells
9. Cover the plate and incubate at room temperature for 15 minutes.
10. Add 50 µL of stop solution to each well and gently mix for 15–20 seconds.
11. Read the absorbance on ELISA Reader for each well at 450 nm within 15 minutes after adding the Stop Solution.

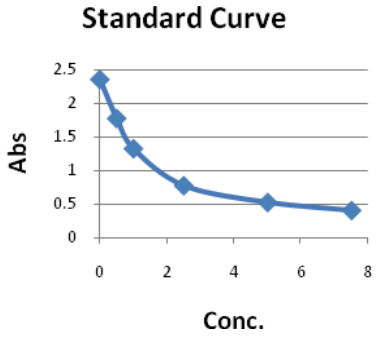
Results

The standard curve is constructed as follows:

1. Check T_3 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 T_3 standards (vertical axis) versus T_3 standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

   Typical Data for a standard Curve

	OD (450 nm)	Concentration (ng/mL)
Std 1	2.35	0
Std 2	1.77	0.5
Std 3	1.32	1.0
Std 4	0.77	2.5
Std 5	0.52	5.0
Std 6	0.40	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

A study of euthyroid adult population was undertaken to determine expected values for the T_3 EIA test system. The mean value, standard deviation, and expected ranges of samples are presented in the following table (total samples tested = 105):

	(105 samples)
Mean	1.184
Standard deviation	0.334
Expected range	0.52–1.85

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. Frank, J.E. et al., Thyroid function in very low birth weight infants: effects on neonatal hypothyroidism screening. J. Pediatr., 1996; 128(4):548-54.
3. Shimada, T. et al., Thyroid functions in patients with various chronic liver diseases. Endocrinol. Jpn., 1988; 35(3):357-69.
4. Thakur, C. et al., Total serum levels of triiodothyronine (T_3) thyroxine (T_4) and thyrotropine (TSH) in school going children of Dibrugarh district: an endemic goitre region of Assam. Indian J. Physiol. Pharmacol., 1997; 41(2):167-70.

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