DRG® Total T3 ELISA (EIA-4569)

CE

USA: RUO

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Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

1 PRINCIPLE

Competitive Enzyme Immunoassay.

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. After equilibrium is attained, the antibody bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

2 REAGENTS

1. **Standard** (Human Serum References) – 0.75 mL/vial
   Six vials of serum reference for triiodothyronine at concentrations of 0 , 0.5 , 1.0 , 2.5 , 5.0 and 10.0 ng/mL.
   Store at 2 °C - 8 °C. A preservative has been added.
2. **Enzyme Conjugate** (ready to use) - 6 mL/vial.
   It contains triiodothyronine-horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix.
   Store at 2 °C - 8 °C.
3. **Assay Reagent** – 6 mL.
   One bottle containing buffer, binding protein inhibitors and anti T3 mAb.
   Store at 2 °C - 8 °C.
4. Antibody Coated **Microplate** – 1 x 96-wells
   coated with goat anti mouse pAb and packaged in an aluminum bag with a drying agent.
   Store at 2 °C - 8 °C.
5. **Wash Solution** Concentrate – 25 mL, 40X concentrated.
   A preservative has been added. Store at 2 °C - 30 °C.
6. **TMB-Substrate** – 1 x 12.0 mL/vial, ready to use.
   Store at 2 °C - 8 °C.
7. **Stop Solution** – 1 x 14.0 mL/vial
   contains 0.5M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.

Note 1: Do not use reagents beyond the kit expiration date.

3 PRECAUTIONS

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

4 SPECIMEN COLLECTION AND PREPARATION

Collect sample(s) by venipuncture in ten (10) mL silicone evacuated tube(s) or evacuated tube(s) containing EDTA or heparin.

The usual precautions in the collection of venipuncture samples should be observed. Separate the red blood cells by centrifugation, use serum or plasma for the total T3 procedure.

Specimen(s) may be refrigerated at 2 °C - 8 °C for a maximum period of 48 hours. If the specimen(s) can not be assayed within 48 hours, the sample(s) may be stored at temperatures of – 20 °C for up to 30 days.

When assayed in duplicate, 0.10 mL (50 µL per test) of the specimen is required.

The cross reactivity of the triiodothyronine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of triiodothyronine needed to displace the same amount of tracer.

Substance	Cross Reactivity (%)
I-Triiodothyronine	100
I-Thyroxine	0.37
Reverse T3	0.75
D-Thyroxine	0.1
3,5-Diiodo-L-Thyrosine	0.2
4-Phenoxyphenol	0.2

5 MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipette capable of delivering 50 µL volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 mL and volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450 nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Quality control materials.
7. Vacuum aspirator (optional) for wash steps.
8. Timer

6 REAGENT PREPARATION:

1. Wash Buffer.

Dilute contents of Wash Concentrate to 1000 mL with distilled or deionized water in a suitable storage container.
Store at room temperature until expiration date printed on concentrate label.

7 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 °C – 27 °C).

1. Format the microplates' wells for each Standard (serum reference), control and sample specimen to be assayed in duplicate.
2. Pipette 50 µL of the appropriate serum reference, (control) or specimen into the assigned well. It is important to add first the standards or sera before adding the assay reagent.
3. Add 50 µL of assay reagent to all wells.
4. Swirl the microplate gently for 10 seconds to mix and cover.
5. **Incubate 30 minutes at room temperature (20 – 27°C).**
6. Add 50 µL of Triiodothyronine-enzyme conjugate solution to all wells.
7. Swirl the microplate gently for 10 seconds to mix and cover.
8. **Incubate 30 minutes at room temperature (20 – 27°C).**
9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
10. Add 300 µL of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes.
    An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.
11. Add 100 µL of TMB-substrate to all wells. Always add reagents in the same order to minimize reaction time differences between wells.
    Incubate 10 minutes at room temperature (20 – 27°C).
    The incubation times are adjusted to 20- 27°C. In case the room temperature is higher than 27°C incubate the TMB-substrate only for 8 minutes (absorption of 0-standard should be not higher than 2.5).
12. Add 100 µL of stop solution to each well. Always add reagents in the same order to minimize reaction time differences between wells.
13. Read the absorbance in each well at 450nm in a microplate reader. The results should be read within ten minutes of adding the stop solution.

Note:
For re-assaying specimens with concentrations more than 10 ng/mL, pipette 25 µL of the specimen and 25 µL of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the triiodothyronine concentration.

8 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the dose response curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

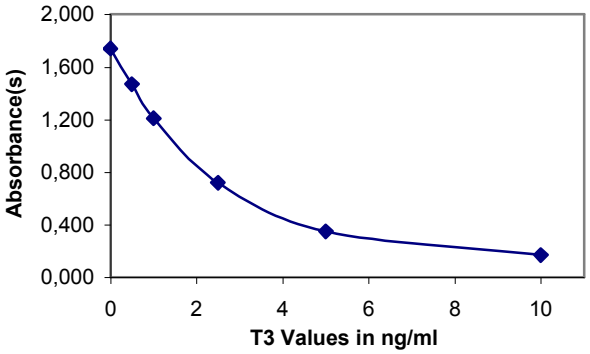
9 RESULTS

A dose response curve is used to ascertain the concentration of triiodothyronine in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding T3 concentration in ng/mL on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of T3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis (y-axis) of the graph, find the intersecting point on the curve, and read the concentration (in ng/mL) from the horizontal axis (X-axis) of the graph (the duplicates of the unknown may be averaged as indicated).

In the following example, the average absorbance 1.208 intersects the dose response curve at 1.06 ng/mL T3 concentration (See Figure 1).

Figure 1



EXAMPLE 1

WELL	SERUM REFERENCES	ABSORBANCE
1	0.0 ng/mL	1.74
2	0.5 ng/mL	1.47
3	1.0 ng/mL	1.21
4	2.5 ng/mL	0.72
5	5.0 ng/mL	0.35
6	10.0 ng/mL	0.17

The data presented in Example 1 and Figure 1 are for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

10 Q.C. PARAMETERS

Maximum Absorbance (Standard 0) = 2.5

11 LIMITATIONS OF PROCEDURE

Assay Performance.

Serum references and controls should not exhibit cloudiness with time. Discard if cloudiness is observed.
Sample(s), which are contaminated microbiologically, should not be used in the assay.
Highly lipemeic or hemolysed specimen(s) should similarly not be used.
It is important that the time of reaction in each well is held constant for reproducible results.

Pipetting of samples should not extend beyond 5 minutes to avoid assay drift. If more than 1 plate is used, it is recommended to repeat the dose response curve.
Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction.
Plate readers measure vertically.
Do not touch the bottom of the wells.
Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
Unused microwell strips should be re-inserted into the aluminum foil bag and re-sealed with the ziploc.

12 REFERENCES / LITERATURE

1. Barker, S.B., "Determination of Protein Bound Iodine." Joumal Biological Chemistry, 173, 175, (1948).
2. Chopra, I.J., Solomon, D.H., and Ho, R.S., "A Radioimmunoassay of Triiodothyronine," J. Clinical EndocrinoL, 33, 865 (1971).
3. Young, D.S., Pestaner, L.C., and Gilberman, U., "Effects of Drugs on Clinical Laboratory Tests." Clinical Chemistry, 21, 3660 (1975).
4. Sterling, L., Diagnosis and Treatment of Thyroid Disease, Cleveland CRC Press, P. 19 51 (1975).