TRIIODOTHYRONINE (T3) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: OKBA00023

Aviva Systems Biology
10211 Pacific Mesa Blvd, Ste 401
San Diego, CA 92121

Enzyme Immunoassay for the Quantitative Determination of Triiodothyronine (T3) in Human Serum

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTENDED USE

The Aviva Total T3 EIA is intended for the quantitative determination of triiodothyronine (T3) concentration in human serum. This test is useful in the research and treatment of thyroid diseases such as hyperthyroidism.

PRINCIPLE OF THE ASSAY

In the Aviva T3 EIA, a second antibody (goat anti-mouse IgG) is coated on a microtiter wells. A measured amount of sample serum, a certain amount of mouse monoclonal Anti-T3 antibody, and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the mouse anti-T3 antibody is bound to the second antibody on the wells. T3 and the enzyme conjugated-T3 compete for the limited binding sites on the anti-T3 antibody. After a 60 minute incubation at room temperature, the wells are washed 5 times by water to remove unbound T3 conjugate. A solution of TMB is then added and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present, and is inversely related to the amount of unlabeled T3 standards assayed in the same way. The concentration of T3 in the unknown sample is then calculated.[7-11]

REAGENTS AND MATERIALS PROVIDED

1. *Antibody-Coated Wells (1 plate, 96 wells)*
   Microtiter wells coated with goat anti-mouse IgG.
2. *11X Enzyme Conjugate Concentrate (1 bottle, 1.3 mL)*
   Contains T3-HRPO Conjugate, with TRIS buffer, pH=7.60 and ProClin-300.
3. *Enzyme Conjugate Diluent (1 bottle, 13 mL)*
   Contains ANS, TRIS buffer, pH=7.60 and ProClin-300.
4. *Reference Standard Set (1 mL/vial)*
   Contains 0, 0.75, 1.5, 3.0, 6.0 and 10.0 ng/mL – 1 set, 1 mL each
5. *T3 Antibody Reagent (1 bottle, 7 mL)*
   Contains mouse monoclonal anti-T3 in phosphate buffer, pH=7.60 and ProClin-300.
7. *TMB Reagent (1 bottle, 11 mL)*
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
8. *Stop Solution (1N HCl) (1 bottle, 11 mL)*
   Contains diluted hydrochloric acid.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes: 25 µL, 100 µL, 200 µL, and 1 mL
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm.
5. Absorbent paper
6. Graph paper
7. Vortex mixer or equivalent

WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[12]
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
6. Do not pipette reagents by mouth.
7. For research purposes only.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

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REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. To prepare **Working T3-HRPO Conjugate Reagent:** add 0.1 mL of T3-HRPO Conjugate Concentrate (11x) to 1.0 mL of T3 Conjugate Diluent (1:11 dilution), and mix well.
   Note: Prepare only the amount of Conjugate that is required each time. Working Conjugate Reagent should be used within 24 hours. Discard the excess after use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Pipette 50µL of standards, specimens, and controls into appropriate wells.
3. Dispense 50µL of T3 Antibody Reagent into each well. Mix thoroughly for 30 seconds.
4. Add 100 µL of ***Working Conjugate Reagent*** into each well. Mix thoroughly for 30 seconds. ***It is important to have complete mixing in step 3 and 4.***
5. Incubate at room temperature (18-25°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µL of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature, in the dark, for 20 minutes.
11. Stop the reaction by adding 100µL of Stop Solution to each well.
12. Gently mix for 30 seconds. ***Ensure that all of the blue color changes completely to yellow.***
13. Read absorbance at 450nm with a microtiter plate reader **within 15 minutes**.

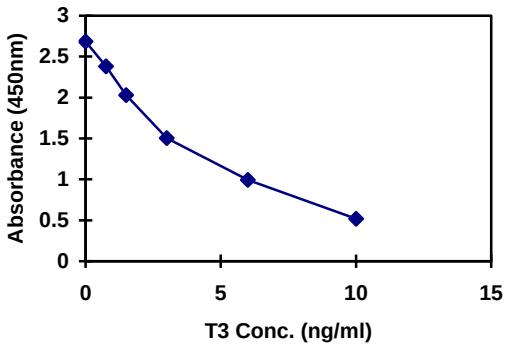
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD_{450}) from the duplicate set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of T3 in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown on the Y-axis against Total T3 concentrations (ng/mL) shown on the X-axis, are presented below. **NOTE:** the standard curve is for illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve for each assay run. Additionally, the absorbance (450nm) values can be varied due to incubation at different room temperature in different laboratories.

Total T3 (ng/mL)	Absorbance (450nm)
0.0	2.685
0.75	2.381
1.5	2.028
3.0	1.502
6.0	0.992
10.0	0.518



TECHNICAL CONSULTATION

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