EAGLE BIOSCIENCES

Free Triiodothyronine (fT3) ELISA Assay Kit

Catalog Number:
T3F31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 3.0 (22 FEB 24)

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INTENDED USE

The Eagle Biosciences Free Triiodothyronine (fT3) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the direct quantitative determination of free triiodothyronine in human serum. The Eagle Biosciences Free Triiodothyronine (fT3) ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Triiodothyronine (T3) is a thyroid hormone found circulating in the bloodstream. T3 contains three iodine atoms and is produced largely through the extrathyroidal conversion of thyroxine (T4), the principal thyroid hormone with four iodine atoms. Most of the T3 that circulates in the blood is bound to carrier proteins such as TBG, pre-albumin and albumin. The free fraction of T3 (fT3), which represents only 0.25% of the total amount, is considered to be the physiological active fraction. Total T3 levels depend not only on thyroid status and the peripheral conversion of T4 to T3, but also on the concentration of thyroid hormone-binding proteins. Free T3 (fT3) on the other hand, is largely unaffected by variations in these carrier proteins which can occur under conditions such as pregnancy, estrogen therapy and the use of oral contraceptives. Therefore, free T3 typically reflects a patient's actual thyroid status more reliably than total T3. Measurement of free T3 is generally recommended for patients with symptoms of hyperthyroidism as found in Graves' disease, toxic adenoma and toxic multinodular goiter.

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of fT3 in the sample. A set of standards is used to plot a standard curve from which the amount of fT3 in patient samples and controls can be directly read. The labelled T3 (conjugate) employed in this assay system has shown no substantial binding properties towards thyroxine-binding globulin (TBG) or human serum albumin (HSA). The binding sites on the microplates are designed to be of a low binding-capacity in order not to disturb the equilibrium between T3 and its carrying proteins. The assay is carried out under normal physiological conditions of pH, temperature and ionic strength.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    c. Wear protective clothing and disposable gloves.
    d. Wash hands thoroughly after performing the test.
    e. Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.

3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit should be reported as > 40 pg/ml and must not be diluted. Dilution will alter the existing equilibrium and may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.

26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

LIMITATIONS

1. This kit is intended for research use only and should not be used in diagnostic procedures.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non- reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 50, 100, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. A 37°C incubator
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)
6. Automatic plate washer

REAGENTS PROVIDED

1. **Microplate**— Ready To Use
   - Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

2. **(HRP) Conjugate Concentrate** — Requires Preparation X51
   - Contents: fT3-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   - Volume: 0.3 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: Dilute 1:51 in assay buffer before use (eg. 40 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of HRP in 12 mL of assay buffer. Discard any that is left over.

3. **Calibrators** — Ready To Use
   - Contents: Six vials containing fT3 in a human serum-based matrix with a non-mercury preservative. Prepared by spiking serum with a defined quantity of T3.

   * Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 pg/mL	0.5 mL
Calibrator B	2 pg/mL	0.5 mL
Calibrator C	4 pg/mL	0.5 mL
Calibrator D	8 pg/mL	0.5 mL
Calibrator E	16 pg/mL	0.5 mL
Calibrator F	40 pg/mL	0.5 mL

   - Storage: Refrigerate at 2–8°C.
   - Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles

4. **Controls** — Ready To Use
   - Contents: Two vials containing fT3 in a human serum-based matrix with a non-mercury preservative. Prepared by spiking serum with defined quantities of T3. Refer to vial labels for acceptable range.
   - Volume: 0.5 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. **Wash Buffer Concentrate** – Requires Preparation x10
   - Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   - Volume: 50 mL/bottle

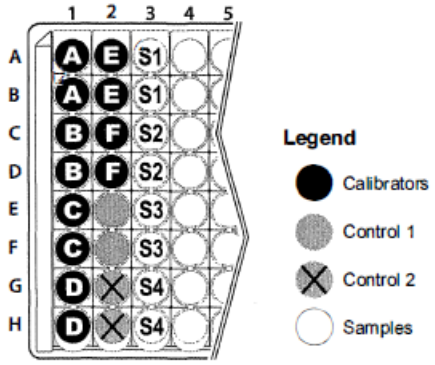
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. **Assay Buffer** – Ready to Use
   Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
   Volume: 15 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

7. **TMB Substrate** – Ready to Use
   Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   Volume: 16 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

8. **Stopping Solution** – Ready to Use
   Contents: One bottle containing 1M sulfuric acid.
   Volume: 6 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

Specimen Pretreatment: None.

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the fT3-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. **Pipette 25 µL** of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. **Pipette 100 µL** of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Gently **tap** the plate for 10 seconds.
6. **Incubate** the plate at 37°C for 1 hour.
7. **Wash** the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
8. **Pipette 150 µL** of TMB substrate into each well at timed intervals.
9. **Incubate** the plate at 37°C for 10–15 minutes (or until calibrator A attains dark blue colour for desired OD).
10. **Pipette 50 µL** of stopping solution into each well at the same timed intervals as in step 8.
11. **Read** the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of calibrator/OD of calibrator A) x 100.

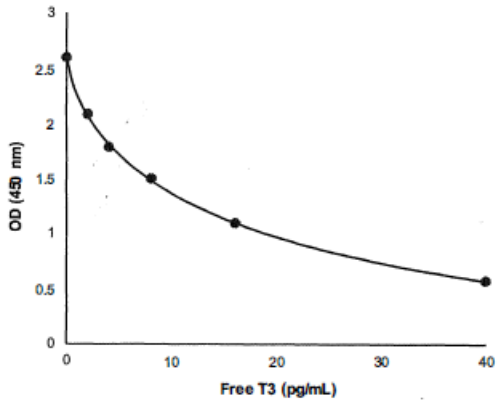
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD	% Binding	Value (pg/mL)
A	2.596	100	0
B	2.082	80	2
C	1.788	69	4
D	1.516	58	8
E	1.024	39	16
F	0.575	22	40
Unknown	2.029	-	4.8

TYPICAL CALIBRATOR CURVE



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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.