EAGLE BIOSCIENCES

Reverse T3 (rT3) ELISA Assay Kit

Catalog Number:
RT331-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 2.2 (29 APR 24)

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INTENDED USE

The Eagle Biosciences Reverse Triiodothyronine (rT3) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the direct quantitative determination of Reverse Triiodothyronine (rT3) in human serum and plasma. The Eagle Biosciences Reverse Triiodothyronine (rT3) ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

3,3′,5′-Triiodo-L-thyronine also known as reverse triiodothyronine or reverse T3 (rT3), differs from 3,3′,5-Triiodo-L-thyronine (T3) in the positions of the iodine atoms in the molecule. The majority of circulatory rT3 is synthesized by peripheral deiodination of thyroxine (T4). Both T3 and rT3 bind to thyroid hormone receptors, but in contrast to T3, rT3 has not been found yet to stimulate receptor metabolic activity; it blocks receptor sites from T3 activation. The ratio of rT3 to T3 is a valuable biomarker of the metabolism and function of thyroid hormones because the process of 5′ monodeiodination that converts T4 to T3 and rT3 to 3,3′-T2 is inhibited in a number of non-thyroidal conditions such as fasting, anorexia nervosa, malnutrition, diabetes mellitus, stress, severe trauma or infection, hemorrhagic shock, hepatic dysfunction, pulmonary diseases and others. This scenario is known as "Sick euthyroid" syndrome or "Low T3" syndrome. An elevated ratio of rT3 over T3 is therefore indicative of "sick euthyroid" syndrome and helps to exclude a diagnosis of hypothyroidism, particularly in critically ill patients 1–9. The concentration of rT3 could be high in patients on the following medications: amiodarone, dexamethasone, propylthiouracil, ipodate, propranolol, and the anesthetic halothane. The concentration of rT3 could be low in patients on Dilantin, which decreases rT3 due to its displacement from thyroxine binding globulin and therefore generates an excessive clearance of rT3.

PRINCIPLE OF THE ASSAY

The rT3 ELISA is a competitive enzyme immunoassay, where the antigen (rT3 present in calibrators, controls and patient samples) competes with a biotin-labelled antigen (rT3-Biotin conjugate) for a limited quantity of antibody which is coated on the microplate wells. After one hour incubation followed by the first washing, unbound materials are removed and a Streptavidin-HRP conjugate is added and incubated for 30 minutes. Following a second washing, the TMB substrate is added. The enzymatic reaction is terminated by addition of the stopping solution, upon which the color intensity is measured with a microplate reader. The color intensity is inversely proportional to the concentration of rT3 in the sample. The set of kit calibrators that are run simultaneously with the samples is used to plot a calibration curve and determine the concentration of rT3 in samples and controls.

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 this 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 saliva 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 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 colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
15. Do not use blood contaminated saliva samples.
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 may be reported as >2 ng/mL. If further dilution and retesting is required, only Calibrator A may be used to dilute samples. The use of any other reagent 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 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 saker used can influence the optical densities and test results. If a different type of shake 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. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
30. 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

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the calibrators 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 other infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

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

Serum: Approximately 0.2 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 -20°C or lower for longer time.

Plasma: Approximately 0.2 mL of plasma is required per duplicate determination. Collect 4–5 mL of blood into EDTA plasma tubes. Store at 4°C for up to 24 hours or at -20°C or lower for longer time.

Consider all human specimens as possible biohazardous materials and take 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)

REAGENTS PROVIDED

1. **Anti-Reverse T3 Polyclonal Antibody-Coated Break- Apart Well 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. **Reverse T3-Biotin Conjugate** – Ready to Use
   - Contents: Reverse T3-Biotin conjugate in a protein-based buffer with a non-mercury preservative.
   - Volume: 13 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

3. **Streptavidin-Horse Radish Peroxidase (HRP) Conjug**ate – Ready to Use
   - Contents: Streptavidin-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   - Volume: 20 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

4. **Reverse T3 Calibrators** — Ready To Use
   - Contents: Six vials containing rT3 in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with rT3 to the concentrations in labels. Typical calibrator concentrations*: 0, 0.02, 0.1, 0.4, 1 and 2 ng/mL.
   - * Approximate value — please refer to vial labels for exact concentrations
   - Volume: Calibrators A-F: 1mL/vial
   - Storage: Refrigerate at 2–8°C.
   - Stability: 12 months in unopened vials or as indicated on label.

5. **Reverse T3 Controls** — Ready To Use
   - Contents: Two vials containing rT3 in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with rT3 to the target concentration in QC certificate. Refer to vial labels for acceptable ranges.
   - Volume: 1 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months in unopened vial or as indicated on label.

6. **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 the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to

be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

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.

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. After all kit components have reached room temperature, mix gently by inversion.
2. Prepare the working wash buffer (see wash buffer concentrate under the section "Reagents Provided").
3. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
4. Pipette **25 µL** of each calibrator, control and specimen sample (serum or plasma) into correspondingly labelled wells in duplicate.
5. Pipette **100 µL** of the Reverse T3-Biotin conjugate into each well (the use of a multichannel pipette is recommended). Gently shake the microplate by hand for ten seconds to ensure complete mixing of the conjugate solution with the calibrators, controls and samples.
6. **Incubate** the plate at 37°C for 1 hour. Do not cover the microplate.
7. **Wash** the wells with 350 µL/well of working wash buffer solution 3 times. After washings tap the plate firmly against absorbent paper to remove any residual liquid. (The use of an automatic strip washer is strongly recommended.)

   The accuracy of this assay depends on the correct execution of the washing procedure.
8. Pipette **150 µL** of the Streptavidin-HRP conjugate into each well. (The use of a multichannel pipette is recommended.)
9. **Incubate** the plate at 37°C for 30 minutes. Do not cover the microplate.
10. **Wash** the wells 3 times using the same procedure as stated in step 6.
11. Pipette **150 µL** of the TMB substrate into each well at timed intervals. (The use of a multichannel pipette is recommended.)

12. **Incubate** at 37°C for 10-20 minutes. Do not cover the microplate.
13. Pipette **50 µL** of stopping solution into each well at the same timed intervals as in step 10 (the use of a multichannel pipette is recommended). Gently shake the microplate by hand for ten seconds to ensure complete mixing of the stopping solution in the wells.
14. **Measure** the absorbance at 450 nm with a microplate reader, within 20 minutes after addition of the stopping solution.

CALCULATIONS

1. Calculate the mean optical density of each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve with immunoassay software to generate the control and sample concentration results or draw a calibration curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis and read the concentration of controls and samples off the calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and calibrator curve.
4. If a sample reads greater than 2 ng/mL report the result as "> 2 ng/mL".
5. To convert from ng/mL to ng/dL multiply the result by 100; to convert to nmol/L, multiply the ng/dL result by 0.01536 or the ng/mL result by 1.536.

QUALITY CONTROL

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

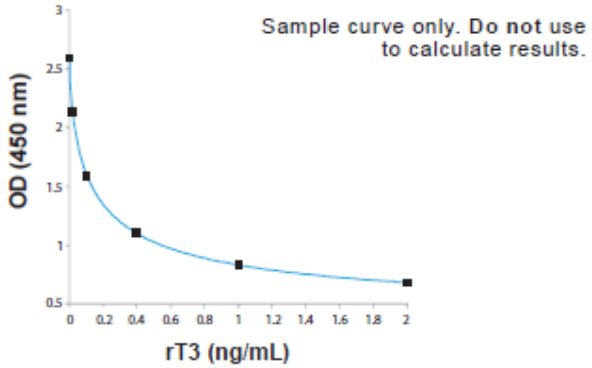
1. The calibrator A mean optical density meets the acceptable range as stated in the QC certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC certificate. % Binding = (OD) of calibrator/OD of calibrator A) x 100
3. The values obtained for 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 (450 nm)	% Binding	rT3 (ng/mL)
A	2.527	100	0
B	2.232	88	0.02
C	1.563	62	0.1
D	0.785	31	0.4
E	0.431	17	1
F	0.270	11	2
Unknown	1.289	-	0

TYPICAL CALIBRATOR CURVE



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

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Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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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.