AccuDiag™ Reverse T3 ELISA Kit

REF 3145-38

IVD See External Label 2°C 8°C 96 Tests

Reverse T3 ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Two-Step Competitive Immunoassay
Sample	25 µL Serum
Incubation Time	110 minutes

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

For the direct quantitative determination of Reverse Triiodothyronine (rT3) in human serum and plasma by an ELISA (Enzyme-Linked Immunosorbent Assay).

For In Vitro Diagnostic Use Only.

LIMITATIONS RELATED TO INTENDED USE

1. This kit is intended for in vitro diagnostic use only.

ASSAY PRINCIPLE

The rT3 ELISA is a two-step competitive immunoassay. In the first incubation step, competition occurs between rT3 present in calibrators, controls, specimen samples and a biotin-labelled antigen (biotin conjugate) for a limited number of anti-rT3 antibody binding sites on the microplate wells. Excess and unbound materials are removed by a washing step. In the second incubation step, streptavidin-HRP (streptavidin HRP conjugate) is added, which binds specifically to any bound biotin conjugate. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-colored product that is inversely proportional to the amount of rT3 present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the color from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of rT3 in specimen samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for in vitro diagnostic use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - 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 and plasma 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. The Biotin Conjugate is sensitive to light and should be a light yellow colour if properly stored. Instability or contamination may be indicated if the solution appears dark green or black in colour, in which case it should not be used.
16. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum and plasma samples.
17. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
18. 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 serum and plasma samples. The use of any other reagent may lead to false results.

19. Avoid microbial contamination of reagents.
20. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
21. To prevent the contamination of reagents, do not pour reagents back into the original containers.
22. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
23. 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.
24. 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.
25. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
26. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
27. 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.
28. Do not reuse the microplate wells, they are for SINGLE USE only.
29. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
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

BIOHAZARDS

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.

The calibrators and controls provided with the kit contain material(s) of human origin that have been tested by approved methods and found to be negative for the presence of HBsAg, HIV-1 (NAT), HCV (NAT), HCV antibody and antibodies to HIV 1/2. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection and Storage

Serum

Approximately 0.2 mL of serum is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labelled tube and allow it to clot at room temperature. Centrifuge at room temperature and carefully transfer the serum into a new labelled storage tube or container.

Serum samples must be stored:
a) Refrigerated (2-8°C) for a period of no longer than 5 days, or
b) Frozen (≤ -20°C) for a period of no longer than 3 months.
Avoid multiple freeze/thaw cycles.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

Plasma

Approximately 0.2 mL of EDTA plasma is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labelled EDTA plasma tube. Centrifuge at room temperature and carefully transfer the plasma into a new labelled storage tube or container.
EDTA plasma samples must be stored:
c) Refrigerated (2-8°C) for a period of no longer than 5 days, or
d) Frozen (≤ -20°C) for a period of no longer than 3 months.
Avoid multiple freeze/thaw cycles.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

Specimen Pre-Treatment and Storage

Do not test samples the same day of the blood draw. The serum or plasma specimen samples must be stored at the recommended storage conditions for at least 20 hours prior to being tested.

REAGENTS

Materials provided with the test kit

1. Microplate

Contents:	One anti-rT3 polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four months.

2. Biotin Conjugate

Contents:	One bottle containing rT3-Biotin conjugate in a protein-based buffer with a non-mercury preservative.
Format:	Ready to Use
Volume:	13 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four months.

3. Streptavidin HRP Conjugate

Contents:	One bottle containing Streptavidin-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Format:	Ready to Use
Volume:	20 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four months.

4. Calibrator A – F

Contents:	Six bottles of calibrator containing specified rT3 concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of rT3. Listed below are approximate concentrations, please refer to vial labels for exact concentrations. Concentrations: 0, 0.02, 0.1, 0.4, 1, 2 ng/mL.
Format:	Ready to Use
Volume:	1.0 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four months.

5. Control 1 – 2

Contents:	Two bottles of control containing different rT3 concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of rT3. Refer to the QC certificate for the target values and acceptable ranges.
Format:	Ready to Use
Volume:	1.0 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four months.

6. TMB Substrate

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Format:	Ready to Use
Volume:	16 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four months.

7. Stopping Solution

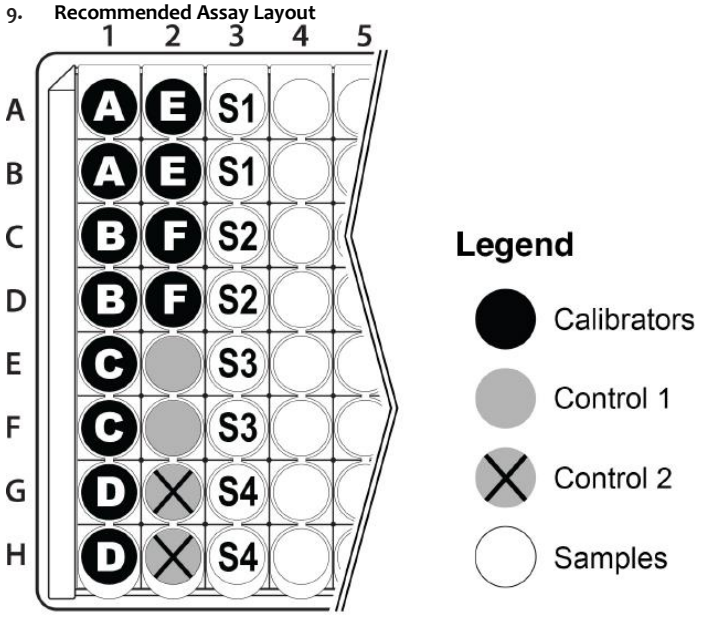
Contents:	One bottle containing 1M sulfuric acid.
Format:	Ready to Use
Volume:	6 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four months.
Safety:	Refer to product SDS. **Warning**

8. Wash Buffer Concentrate

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four months. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.

Preparation of Wash Buffer Working Solution:	(X10) **Dilute 1:10 Before Use** Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

9. Recommended Assay Layout



Materials required but not provided

1. Calibrated single-channel pipette to dispense 25 µL.
2. Calibrated multi-channel pipettes to dispense 50 µL, 100 µL and 150 µL.
3. Calibrated multi-channel pipettes to dispense 350 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Microplate shaker: Orbital shaker (3 mm diameter) set to 600 rpm.
6. Disposable pipette tips.
7. Distilled or deionized water.
8. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.

ASSAY PROCEDURE

Specimen Pre-Treatment:

Do not test samples the same day of the blood draw. The serum or plasma specimen samples must be stored at the recommended storage conditions for at least 20 hours prior to being tested.

All kit components, controls and specimen samples must reach room temperature prior to 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 Wash Buffer Working Solution (See section *Materials provided with the test kit, 8. Wash Buffer Concentrate*).
3. **Plan** the microplate wells to be used for calibrators, controls, and samples. See section *9. Recommended Assay Layout.* Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.

4. **Pipette 25 µL** of each calibrator, control, and specimen sample into assigned wells.
5. **Pipette 100 µL** of the Biotin Conjugate into each well (the use of a multi-channel pipette is recommended).
6. **Incubate** the microplate on a microplate shaker** for **60 minutes** at room temperature.
7. **Wash** the microplate wells with an automatic microplate washer (preferred) or manually as stated below.
   Automatic: Using an automatic microplate washer, perform a **3-cycle** wash using **350 µL/well** of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells then filling each well with 350 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

   Manually: For manual washing, perform a **3-cycle** wash using **350 µL/well** of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 350 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate 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 multi-channel pipette is recommended).
9. **Incubate** the microplate on a microplate shaker** for **30 minutes** at room temperature.
10. **Wash** the microplate wells again as stated in step 7.
11. **Pipette 150 µL** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
12. **Incubate** the microplate on a microplate shaker** for **10-20 minutes** at room temperature.
13. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
14. **Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

** See section *Materials required but not provided* for microplate shaker options.

CALCULATIONS

1. Calculate the mean optical density for each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 2 ng/mL and needs to be diluted and retested, then dilute with calibrator A not more than 1:5. The result obtained must be multiplied by the dilution factor.
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 DATA

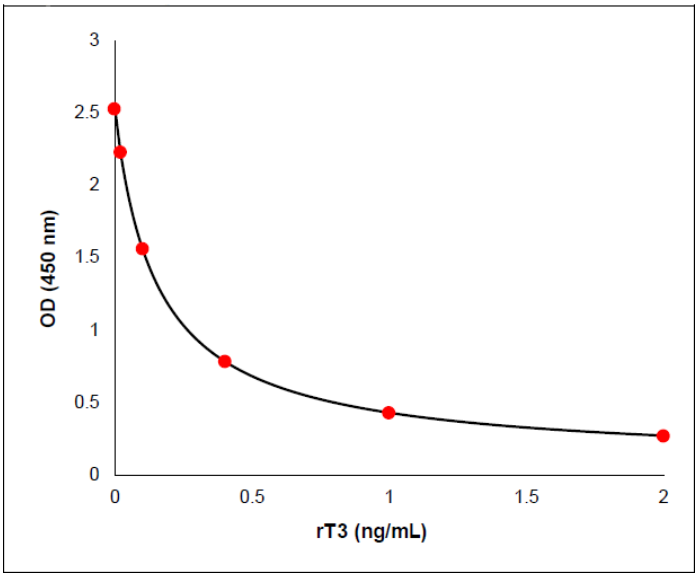
TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (ng/mL)
A	2.527	100	0
B	2.232	88	0.02
C	1.563	62	.01
D	0.785	31	.04
E	0.431	17	1
F	0.270	11	2
Unknown	1.289	-	0.15

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



CHANGE HISTORY

Previous Version:	6.0 (Combined)	New Version:	IVD-7.0
Changes:	**Clinical Applications** section removed. **Performance Characteristics** section removed. **References** section removed. **EC REP** information removed.		

Previous Version:	6.0 (Combined)	New Version:	IVD-7.0
	New IFU format for headings. **HEADING** Removal of country-specific regulatory information and addition of IVD symbol. **INTENDED PURPOSE & USE** Addition: For In Vitro Diagnostic Use Only. **LIMITATIONS RELATED TO INTENDED PURPOSE & USE** Replaced all limitations with the following statement: This kit is intended forin vitro diagnostic use only. **PROCEDURAL CAUTIONS AND WARNINGS** Additional cautions and warnings added. Some previous limitations added to this section. **REAGENTS PROVIDED** Addition of safety information for components if applicable. In-use stability statement added for all components. Control low and high now called control 1 and 2, respectively. **RECOMMENDED ASSAY LAYOUT** New section added. **ASSAY PROCEDURE** Component names revised to match symbol definitions. **QUALITY CONTROL** New section added. **CHANGE HISTORY** New section added. **GENERAL INFORMATION** Addition of product complaints, warranty and limitation of liability sections.		

PRODUCT COMPLAINTS

In the case of product complaints, the user shall submit in writing to the distributor or manufacturer a description of the complaint and provide accompanying data and/or information.

WARRANTY

Diagnostic Automation Inc. guarantees that the product is free of defects and will perform within the product specifications when the product is used prior to the expiration date, according to the intended purpose and use, and according to the instructions for use provided with the product. Any deviations from the intended purpose and use, instructions for use, modifications to kit components or use beyond the expiration date will invalidate any warranty claims.

LIMITATION OF LIABILITY

Diagnostic Automation Inc. liability in all circumstances whether in tort (including negligence) or at common law, and for any damage or loss, including but not limited to loss of profit and loss of sales, suffered whether direct, indirect, consequential, incidental or special is limited to the purchase price of the product(s) in question.

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

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Date Adopted	**2022-09**
REF **3145-38**	**AccuDiag™ - Reverse T3 ELISA**
Brand Name	**AccuDiag™**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands.** www.cepartner4u.eu
Revision Date: 2023-Feb-28	