Reverse T3 ELISA Kit

Reverse T3 ELISA Kit is an enzyme immunoassay kit for the quantification of Reverse T3 in human serum and plasma.

Catalog number: ARG83086

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Reverse T3 (Reverse triiodothyronine, rT3) is an isomer of triiodothyronine.

Reverse T3 is the third-most common iodothyronine the thyroid gland releases into the bloodstream, at 0.9%; tetraiodothyronine constitutes 90% and T3 is 9%. However, 95% of rT3 in human blood is made elsewhere in the body, as enzymes remove a particular iodine atom from T4.

The production of hormone by the thyroid gland is controlled by the hypothalamus and pituitary gland. The physiological activity of thyroid hormone is regulated by a system of enzymes that activate, inactivate or simply discard the prohormone T4 and in turn functionally modify T3 and rT3. These enzymes operate under complex direction of systems including neurotransmitters, hormones, markers of metabolism and immunological signals.

The levels of rT3 increase in conditions such as euthyroid sick syndrome because its clearance decreases while its production stays the same. The decreased clearance is possibly from lower Thyroxine 5-deiodinase activity in the peripheral tissue or decreased liver uptake of rT3. In addition, increased rT3 concentrations result from upregulated Thyroxine 5-deiodinase activity in critical illness, starvation and fetal life.

PRINCIPLE OF THE ASSAY

The rT3 ELISA is a competitive enzyme immunoassay, where the antigen (rT3 present in standards, controls and 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 Standards that are run simultaneously with the samples is used to plot a standard curve and determine the concentration of rT3 in samples and controls.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C
Standard A-F (0, 0.02, 0.1, 0.4, 1, 2 ng/ml)	6 x 1 ml/vials	4°C
Control 1 and 2	2 x 1 ml/vials	4°C
rT3-Biotin conjugate	13 ml	4°C
HRP-Conjugate	20 ml (Ready-to-use)	4°C
10X Wash Buffer	50 ml	4°C
TMB Substrate	16 ml (Ready-to-use)	4°C
Stop Solution	6 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620-650 nm as reference wavelength)
- Microplate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.

- Change pipette tips between the addition of different reagent or samples.
- Store the unopened reagents at 2 -8°C until expiration date. Once opened the reagents are stable for 1 month when stored at 2-8°C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at-20 or-70°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. Storage: up to 1 months at 2-8°C.

ASSAY PROCEDURE

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **25 µl** extracted **standards**, **controls** or **samples** into wells.
3. Add **100 µl rT3-Biotin conjugate** into each wells.
4. Incubate **60 mins** at RT.
5. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **150 µl** of **HRP-Conjugate** into each wells.
7. Incubate for **30 mins** at RT.
8. Aspirate each well and **wash** as step 4.
9. Add **150 µl** of **TMB substrate solution** into each well. Incubate for **10-20 mins** at RT in dark.
10. Add **50 µl** of **Stop Solution** to each well and shake lightly to ensure homogeneous mixing.
11. Read the OD with a microplate reader at **450nm** (with a reference wavelength between 620nm and 650nm) within 15 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

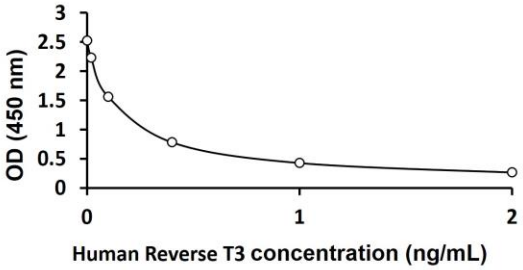
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

0.014 ng/ml

Assay Range

0.02-2 ng/ml

Specificity

Not reacts with Not reacts withT3, T4 and T2.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was < 5.0% and CV value of inter-assay precision was < 8.5%.