Diagnostics Biochem Canada

Reverse Triiodothyronine ELISA

REF CAN-RT3-100

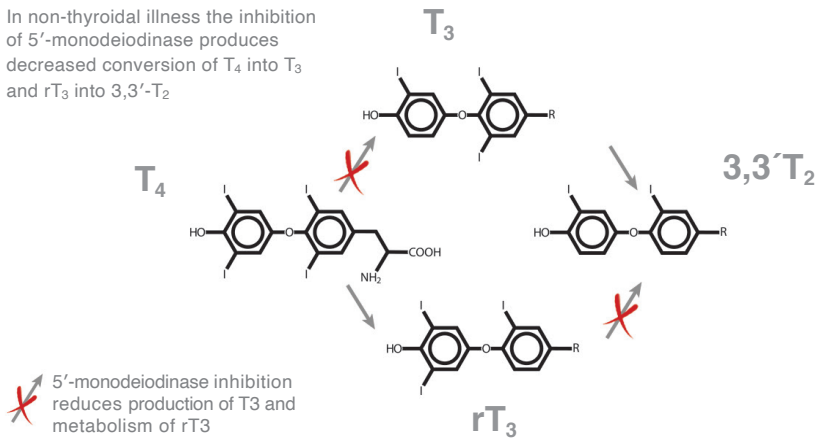
ASSAY PROCEDURE

- Bring kit components to room temperature. Prepare working wash buffer.
- Pipette 25 µL of each calibrator, control and specimen sample.
- Pipette 100 µL of RT3-Biotin conjugate into each well.
- Incubate on a microplate shaker for 1 hour at room temperature. Wash 3 times.
- Pipette 150 µL of Streptavidin-HRP conjugate.
- Incubate on a microplate shaker for 30 minutes at room temperature. Wash 3 times.
- Pipette 150 µL of TMB substrate.
- Incubate on a microplate shaker for 10–20 minutes at room temperature.
- Pipette 50 µL of stopping solution. Gently shake the microplate by hand for 10 seconds.
- Read the plate on a microplate reader at 450 nm.

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 de-iodination 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. 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 TEST

The DBC 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.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of at least 60 samples of the blank and a low value sample in two independent experiments and it was calculated as follows:
LoD = μB + 1.645σB + 1.645σS, where σB and σS are the standard deviation of the blank and low value sample and μB is the mean value of the blank.

The Limit of Detection (LoD) was determined to be **0.014 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with rT3 cross-reacting at 100%:

Steroid	% Cross-Reactivity
rT3	100
T3	0
T4	< 0.1
T2	0

INTERFERENT SUBSTANCES

The following substances did not show significant interference with the assay: hemoglobin up to 2 g/L, free and conjugated bilirubin up to 200 mg/L, triglycerides up to 5.0 mg/mL and Biotin up to 2.4 μg/mL.

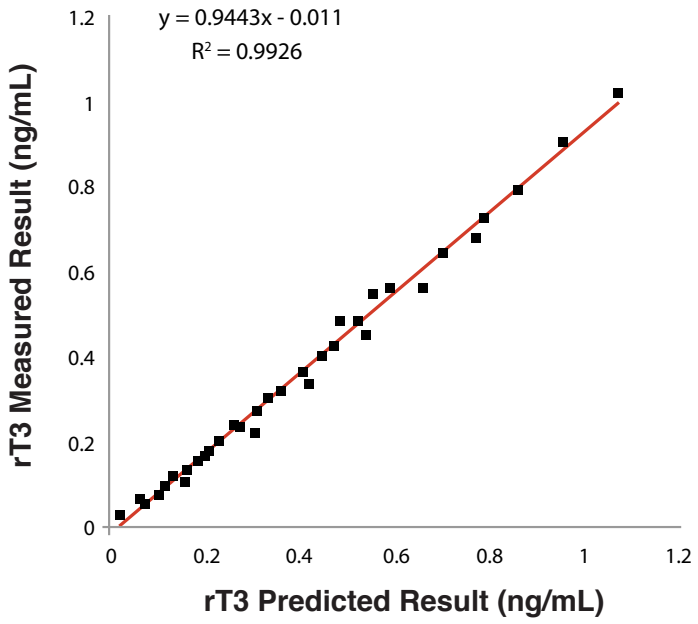
PRECISION

The experimental protocol used a nested components-of-variance design with 2 testing days, two lots and five scientists per day, each scientist ran 2 tests (one test with each lot) per day, and two replicate measurements per run (a 2 x 5 x 2 x 2 design) for each sample. The esults were analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean (ng/mL)	Within Run SD	Within Run CV (%)	Total SD	Total CV (%)
1	0.233	0.01	3.6	0.02	7.0
2	0.535	0.01	2.4	0.05	9.5
3	1.174	0.06	4.9	0.13	10.7
4	0.102	0.01	6.0	0.01	10.4
5	0.082	0.00	5.8	0.01	8.7
6	0.094	0.01	8.1	0.01	11.5
7	0.257	0.01	4.3	0.02	9.7
8	0.480	0.02	4.2	0.04	9.2

LINEARITY

The linearity study was performed with four human serum samples covering the range of the assay and following CLSI guideline EP6-A. The samples were diluted in calibrator A up to a 1:5 dilution, tested in duplicate, and the results compared to the predicted concentration. The statistical analysis shows that the assay is sufficiently linear.



COMPARATIVE STUDIES

The DBC rT3 ELISA kit (y) was tested manually, as well as with automated technology. The comparison of 40 samples yielded the following linear regression results:

y = 0.8452x + 0.0195, r = 0.96

REFERENCE VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Median (ng/mL)	95% Range (ng/mL)	Total Range (ng/mL)
Serum	120	0.15	0.098–0.218	0.069–0.262
Plasma	120	0.15	0.098–0.26	0.072–0.309

Ordering Information: REF CAN-RT3-100

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