Human T4 / Thyroxine (total) ELISA Kit

Enzyme Immunoassay for the quantification of T4 / Thyroxine (total) in Human serum and plasma (EDTA, lithium heparin, citrate).

Catalog number: ARG82802

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Thyroid hormones are two hormones produced and released by the thyroid gland, namely triiodothyronine (T3) and thyroxine (T4). They are tyrosine-based hormones that are primarily responsible for regulation of metabolism. T3 and T4 are partially composed of iodine. A deficiency of iodine leads to decreased production of T3 and T4, enlarges the thyroid tissue and will cause the disease known as simple goitre. The major form of thyroid hormone in the blood is thyroxine (T4), which has a longer half-life than T3. In humans, the ratio of T4 to T3 released into the blood is approximately 14:1. T4 is converted to the active T3 (three to four times more potent than T4) within cells by deiodinases (5′-iodinase). These are further processed by decarboxylation and deiodination to produce iodothyronamine (T1a) and thyronamine (T0a). All three isoforms of the deiodinases are selenium-containing enzymes, thus dietary selenium is essential for T3 production.

Edward Calvin Kendall was responsible for the isolation of thyroxine in 1915. In 2018, levothyroxine, a manufactured form of thyroxine, was the second most commonly prescribed medication in the United States, with more than 105 million prescriptions. Levothyroxine is on the World Health Organization's List of Essential Medicines. [Provided by Wikipedia: Thyroid hormones]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. A highly specific antibody for Thyroxine (T4) has been pre-coated onto a microplate. Total Thyroxine containing samples, Controls or Standards and T4-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and Triiodothyronine compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted Wash Buffer to remove unbound material. Then TMB substrate is added to the wells and color develops in inversely proportion to the amount of T4 present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of T4 in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standards A to F (0, 25, 50, 100, 175, 250 nmol/L)	0.5 mL each (ready to use)	4°C
Control 1 & 2	0.5 mL each (ready to use)	4°C
T4-HRP Conjugate	12 mL (ready to use)	4°C
40X Wash Buffer	30 mL	4°C
TMB substrate	12 mL (ready to use)	4°C (protect from light)
STOP solution	14 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Microplate shaker
- Pipettes and pipette tips
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (18-25°C) before use.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 40X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve and controls each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Plasma: Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. Collect the plasma layer and store on ice.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Samples containing sodium azide should not be used in the assay.
4. Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. Specimens stored for a longer time (up to 8 months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.
5. If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Standard A and re-assayed. For the calculation of the concentrations this dilution factor has to be taken into account.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute 40X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 30 mL of 40X Wash Buffer into 1170 mL of distilled water to a final volume of 1200 mL) The 1X Wash Buffer is stable for up to 2 weeks at room temperature.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 18-25°C) before use. Standards and samples should be assayed in duplicates.

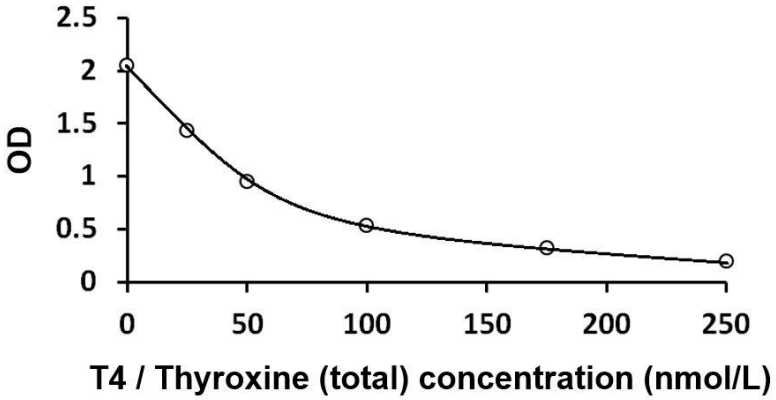
1. Add **10 µL** of **Standards, Controls and samples** into the appropriate wells of the Antibody Coated Microplate.
2. Incubate at **RT** for **5 minutes**.
3. Add **100 µL** of **T4-HRP Conjugate** into all wells. Thoroughly mix for **10 seconds.** It is important to have a complete mixing in this step.
4. Incubate at **RT** for **80 minutes** on a microplate shaker.
5. Aspirate each well and wash, repeating the process 4 times for a total **5 washes.** Wash by filling each well with **1× Wash Buffer (400 µL)** using a squirt bottle, manifold dispenser, or autowasher. Then 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 **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate in the dark for **10 minutes** at **RT** on a microplate shaker.
7. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank

wells. The color of the solution should change from blue to yellow.

8. Read the OD with a microplate reader at 450 nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 10 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human T4 / Thyroxine (total) ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards 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. 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.
4. 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)
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 250 nmol/L. For the calculation of the concentrations this dilution factor has to be taken into account.

QUALITY ASSURANCE

Sensitivity

The sensitivity of the Human T4 / Thyroxine (total) ELISA kit is 8.0 nmol/L.

Specificity

Substance	Cross Reactivity (%)
T3 (3,3`,5-triiodothyronine)	1.5
rT3 (3,3`,5-triiodothyronine, reverse T3)	1.5
3,5-Diiodothyronine	< 0.1

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.6-5.2% and CV value of inter-assay precision was 4.9-8.1%.

Recovery

Samples have been spiked by adding T3 solutions with known concentrations in a 1:1 ratio.

		Sample 1	Sample 2	Sample 3
Conc. (nmol / L)		42.8	79.7	115.0
Average Recovery (%		99.3	102.0	105.3
Range of Recovery (%)	From	97.0	96.5	103.9
	To	101.6	106.6	107.3

Expected Normal Values

Population	Valid N	Range (nmol/L)	Mean (nmol/L)
Adults	115	51.2-159.3	87.2