Human T4 / Thyroxine (free) ELISA Kit

Human T4 / Thyroxine (free) ELISA Kit is an enzyme immunoassay kit for the quantification determination T4 / Thyroxine (free) concentration in Human serum or plasma (EDTA, heparin or citrate plasma)

Catalog number: ARG82906

Package: 96 wells

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MANUFACTURED BY:

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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'-deiodinase). These are further processed by decarboxylation and deiodination to produce iodothyronamine (T_1a) and thyronamine (T_0a). All three isoforms of the deiodinases are selenium-containing enzymes, thus dietary selenium is essential for T3 production.

American chemist 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. Thyroxine (T4) containing samples, Controls or Standards and a Thyroxine (T4) -HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and T4 / Thyroxine (free) compete for the antibody binding sites. After incubation, 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 / Thyroxine (free) 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 / Thyroxine (free) 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. Microtiter wells must be stored at 2-8°C.

Once the foil bag has been opened, care should be taken to close it tightly again.

Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standards 0 to 5 (0, 0.5, 1.0, 2.0, 4.0, 8.0 ng/dL)	1 mL each (ready to use)	4°C
Control 1	1 mL (ready to use)	4°C
Control 2	1 mL (ready to use)	4°C
Thyroxine (T4)-HRP Conjugate	14 mL (ready to use)	4°C
40X Wash Buffer	30 mL	4°C
TMB substrate	25 mL (ready to use)	4°C (protect from light)
STOP solution	14 mL (ready to use)	4°C

Note:

1. Additional Standard 0 for sample dilution is available upon request.
2. Conversion: 1 ng/dL x 12.9 = pmol/L.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm
- Deionized or distilled water
- 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. Opened reagents must be stored at 2-8 °C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 8 weeks if stored as described above.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Bring all reagents and required number of strips to room temperature prior to use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents must be mixed without foaming before use.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- Once the test has been started, all steps should be completed without interruption.
- 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.

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. Patients receiving anticoagulant therapy may require increased clotting time. Remove serum and assay immediately. The samples can be stored at 2-8 °C up to 7 days or aliquot and store samples at ≤ -20 °C or up to one month. And the sample at -20°C should be frozen only once. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA, citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately. The samples can be stored at 2-8 °C up to 7 days or aliquot and store samples at ≤ -20 °C or lower for up to one month. And the sample at -20°C should be frozen only once. Avoid repeated freeze-thaw cycles.

Note:

- Do not use haemolytic, icteric or lipaemic specimens.
- Thawed samples should be inverted several times prior to testing.
- Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. E.g. Add 30 ml of 40 X Wash buffer into 1170 ml of distilled water to a final volume of 1200 ml. The diluted 1X Wash buffer is stable for 2 weeks at room temperature.
- Samples: In an initial assay, if a specimen is found to contain more than the highest standard, the specimens can be diluted with Standard 0 and re-assay. For the calculation of concentration this dilution factor has to be taken into account.

Example:

a) Dilution 1:10: 10 µL sample + 90 µL Standard 0 (mix thoroughly)

b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Standard 0 (mix thoroughly).

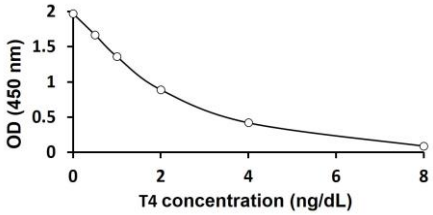
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **50 µL** of **Standards, Controls and prepared samples** into the appropriate wells of the Antibody Coated Microplate.
2. Add **100 µL** of **Thyroxine (T4)-HRP Conjugate** into all wells. Thoroughly mix for **10 seconds**. It is important to have a complete mixing in this step.
3. Incubate at **RT** for **60 minutes**.
4. Aspirate each well and wash, repeating the process 3 times for a total **4 washes**. Wash by filling each well with **1× Wash Buffer** (400 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.
5. Add **150 µL** of **TMB Substrate** to each well, including the blank wells. Incubate at **RT** for **30 minutes** in the dark.
6. 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.
7. Read the OD with a microplate reader at **450 nm** immediately. 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 (free) 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 > 8 ng/dL. For the calculation of the concentrations this dilution factor has to be taken into account.
6. Expected normal value:

Population	n	Mean (ng/dL)	Median (ng/dL)	Range (min. – max.) (ng/dL)
Males	60	1.03	0.96	0.68 – 1.88
Females	60	0.93	0.89	0.66 – 1.79

QUALITY ASSURANCE

Sensitivity

The sensitivity of the Human T4 / Thyroxine (free) ELISA kit is 0.22 ng/dL.

The Limit of Blank (LoB) is 0.20 ng/dL.

The Limit of Detection (LoD) is 0.271 ng/dL.

The Limit of Quantification (LoQ) is 0.298 ng/dL.

Specificity

Substance	Cross Reactivity (%)
T4	0.03
T3	0.01

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was ≤ 7.2% and CV value of inter-assay precision was ≤ 6.9%.

Recovery

85.0-114.8%