



KAMIYA BIOMEDICAL COMPANY

Human Thyroxine (T4) ELISA

For the quantitative determination of human T4 in serum.

Cat. No. KT-29979

For Research Use Only. Not for use in diagnostic procedures.

Product Information
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INTENDED USE

This ELISA kit is a sandwich enzyme immunoassay for the *in vitro* quantitative measurement of human T4 in serum. For research use only. Not for use in diagnostic procedures.

COMPONENTS

Reagents	Quantity
Pre-coated, ready to use 96-well plate	1
Calibrator- 1, 2, 3, 4, 5, 6	6
Detection Reagent A	1 × 6 mL
Detection Reagent B	1 × 6 mL
TMB Substrate	1 × 9 mL
Stop Solution	1 × 6 mL
Wash Buffer (20X concentrate)	1 × 15 mL
Plate sealer for 96 wells	4

Calibrator (ng/mL)

Calibrator 1	Calibrator 2	Calibrator 3	Calibrator 4	Calibrator 5	Calibrator 6
0	20	40	80	160	320

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader with 450 ± 10 nm filter.
2. Precision single and multi-channel pipettes and disposable tips.
3. Eppendorf Tubes for diluting samples.
4. Deionized or distilled water.
5. Absorbent paper for blotting the microtiter plate.
6. Container for Wash Solution

STORAGE

All reagents should be stored according to their label. The kits should be stored at 4°C upon receipt. The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. Opened test kits will remain stable until the expiration date, provided they are stored as above.

PRINCIPLE

This assay employs a competitive inhibition enzyme immunoassay technique. A polyclonal antibody specific for human T4 has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled human T4 (calibrators and samples) with the pre-coated antibody specific for human T4. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well and a change in color will be exhibited. The greater the amount of human T4 in samples, the less the biotin labeled

human T4 bound by pre-coated antibody. The color develops in opposition to the amount of T4 bound in the initial step. Color development is stopped and color intensity is measured.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000 x g. Assay freshly prepared serum immediately or store samples in aliquots at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.



Note:

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. Before performing the assay, slowly bring samples to room temperature.
3. Avoid hemolysis as excessive hemolysis will impact the result.

REAGENT PREPARATION

Bring all kit components and samples to room temperature (18-25°C) before use.

Calibrators

The concentrations of the calibrators are as follows: 320, 160, 80, 40, 20, and 0 ng/mL.

Wash Solution

Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water to prepare 300 mL of Wash Solution (1X).

TMB Substrate

Aspirate the needed dosage of the solution with sterilized tips and do not return the residual solution to the vial.

ASSAY PROCEDURE

Estimate the sample T4 concentration before assaying. If the estimated values are not within the range of the calibration curve, users must determine the optimal sample dilutions for their particular experiments.

1. Determine wells for diluted calibrator, blank and sample. Prepare 5 wells for calibrator, 1 well for blank. Add 50 μ L each of dilutions of calibrator (read Reagent Preparation), blank and samples into the appropriate wells. Then add 50 μ L of Detection Reagent A to each well immediately. Shake plate gently. Cover with the Plate sealer. Incubate for 1 hour at 37°C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
2. Aspirate the solution and wash with 300-350 μ L of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3-5 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 50 μ L of **Detection Reagent B** working solution to each well. Incubate for 30 minutes at 37°C after covering it with the Plate sealer.
4. Repeat the aspiration/wash process for 3-5 times as conducted in step 2.
5. Add 90 μ L of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate for 15 minutes at 37°C. Protect from light. The liquid will turn blue by the addition of Substrate Solution.

6. Add 50 μ L of **Stop Solution** to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.

**Note:**

1. **Assay preparation:** Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Unused strips should be resealed and stored at -20°C until the expiration date.
2. **Sample or reagent additions:** Use freshly prepared Calibrators. Carefully add samples to wells and mix gently to avoid foaming. Do not touch the well walls if possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all calibrators and samples, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each calibrator level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. **Incubation:** To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. After reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. **Washing:** The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drops of solution or fingerprints on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading.
5. **Controlling of reaction time:** Observe the change of color after adding **TMB Substrate** (e.g. observation once every 10 minutes), if the color is too dark, add **Stop Solution** in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. **TMB Substrate** is easily contaminated. Protect from light.

CALCULATION OF RESULTS

This assay employs a competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between T4 concentration in the sample and the assay signal intensity. Low levels of T4 result in a high OD value, while high concentrations result in a low signal.

Average the duplicate readings for each calibrator, control, and samples. Create a calibration curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a calibration curve by plotting the mean absorbance for each calibrator on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph (5 points). The data may be linearized by plotting the log of the T4 concentrations versus the log of the OD and the best fit line can be determined by regression analysis. Using some plot software is also recommended. If samples have been diluted, the concentration read from the calibration curve must be multiplied by the dilution factor.

PERFORMANCE

Detection Range

The detection range is: 20-320 ng/mL.

The calibration curve concentrations used for the ELISA's 320, 160, 80, 40, 20 ng/mL.

Sensitivity

The minimum detectable dose of Human T4 is typically less than 10 ng/mL. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by the mean O.D. value of 20 replicates of the zero calibrator plus three standard deviations.

Specificity

This assay has high sensitivity and excellent specificity for detection of Human T4. No cross-reactivity with T3 was observed.

IMPORTANT NOTES

1. The final experimental results will be closely related to the operative skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
2. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. However, until all factors have been tested the possibility of interference cannot be eliminated.
3. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
4. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be tightly closed to prevent the evaporation or contamination by microorganisms.
5. There may be some foggy substance in the plate wells when first opened. This will have no effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.
6. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 O.D. or greater at 450nm wavelength is acceptable for use in absorbance measurement.
7. The Stop Solution used with this kit is an acid. Wear eye, hand, face, and clothing protection when using this material.

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