Technical Manual

General Thyroxine (T4) ELISA Kit

- Catalogue Code: UNDL00072
- Competitive ELISA Kit
- Research Use Only

DOMINIQUE DUTSCHER SAS

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1. Key Features and Sample Types

Aliases:

3,5,3',5'-Tetraiodothyronine,L-Thyroxine,Levothyroxine

Detection method:

Competitive Inhibition

Sample Type:

serum, plasma and other biological fluids.

Reactivity:

General

Range:

1.25-80ng/mL

Sensitivity:

0.63ng/mL

Storage:

2-8°C for 12 months

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C. Date of expiration will be on the ELISA Box label.

- **Unopened Kits:** All the reagents should be kept according to labels on the vials. The TMB Substrate, Wash Buffer (30X concentrate) and the Stop Solution should be stored at 4°C upon receipt while the others should be at -20°C.
- **Opened Kits:** Once the kit is opened, the remaining reagents still need to be stored according to the above storage conditions. In addition, return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal.

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3. Description and Principle

The Assay Genie Sandwich ELISA kit is a highly sensitive assay for the quantitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ELISA kits work?

The Assay Genie (enzyme-linked immunosorbent assays) assay kits are designed for the quantitative measurement of analytes in a wide variety of samples. As today's scientists demand premium quality, consistent data, Assay Genie have developed a range of sensitive, fast and reliable ELISA kit assays to meet and exceed those demands. Our assay kits use a quantitative sandwich ELISA technique and each kit comes with highly specific antibodies precoated onto a 96-well microtiter plate.

This Assay Genie (enzyme-linked immunosorbent assays) assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to T4 has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labelled T4 and unlabelled T4 (Standards or samples) with the pre-coated antibody specific to T4. After incubation, the unbound conjugate is washed off. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of T4 in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of T4 in the sample.

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4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-Well Kit	Storage
1	Pre-Coated ELISA Microplate	1	-20°C
2	Lyophilised Standard	2 vials	-20°C
3	Diluent Buffer	45 mL	-20°C
4	Detection Reagent A	70 uL	-20°C
5	Detection Reagent B	120 uL	-20°C
6	TMB Substrate	9 mL	2-8°C
7	Stop Solution	6 mL	2-8°C
8	Wash Buffer (30x)	20 mL	2-8°C
9	Plate Sealer	2 pieces	
10	Manual	1	

Additional materials required:

1. Microplate reader with 450 ± 10nm filter.
2. Precision single or 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.

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Precautions:

1. Do not perform a serial dilution directly in the wells.

2. Prepare standard within 15 minutes of performing the assay. Do not dissolve the reagents at 37˚C directly.

3. Detection Reagent A and B are sticky solutions, therefore slowly pipette them to reduce the volume errors.

4. Carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to pipette more than 10μL at a time to ensure accuracy.

5. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.

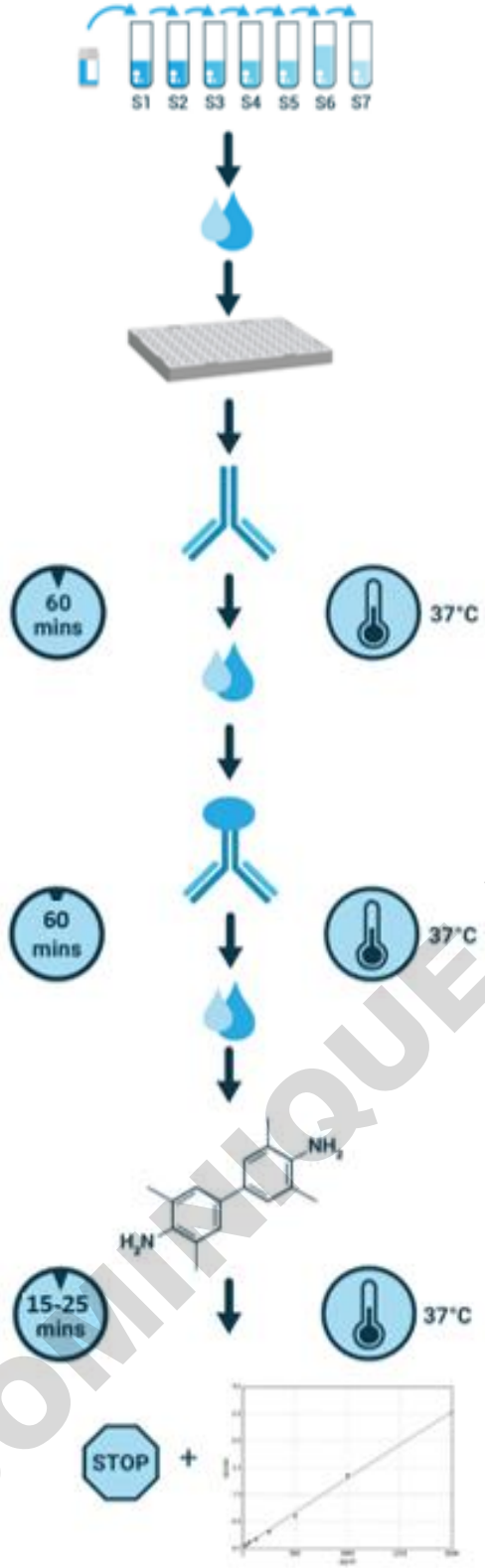
6. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.

7. Any contaminated water or container used during reagent preparation will influence the detection result.

8. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this reagent.

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5. Workflow Overview



Prepare plate standards and reagents

Add 50µl of samples, standards and controls. Immediately add 50µl Detection Reagent A

Wash plate x3

Add 100µl Detection Reagent B

Wash plate x 5

Add 90µl TMB Substrate

Add 50µl STOP solution and read absorbance @ 450nm

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6. Sample Preparation

General considerations:

1. Assay Genie is only responsible for the kit itself, not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. Samples should be diluted by 0.01mol/L PBS (pH=7.0-7.2).
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Tissue or cell extraction samples prepared using a chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.
5. Due to the possibility of mismatching between antigens from other origin and antibodies used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
6. Samples from cell culture supernatant may not be detected by the kit due to influence from factors such as cell viability, cell number and/or sampling time.
7. Fresh samples that have not been stored for extended periods of time are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and give inaccurate or incorrect results.

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Sample Collection and Storage

Serum: Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000 × 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.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8˚C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquots at -20˚C or -80˚C for later use. Avoid repeated freeze/thaw cycles.

Other Biological Fluids: Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquots at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Notes

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/or contamination.

2. Noticeable hemolysis will affect antibody-antigen reactions. Samples with any sign of hemolysis are not acceptable for this assay.

3. When performing the assay, bring samples to room temperature.

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7. Standard and Reagent Preparation

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

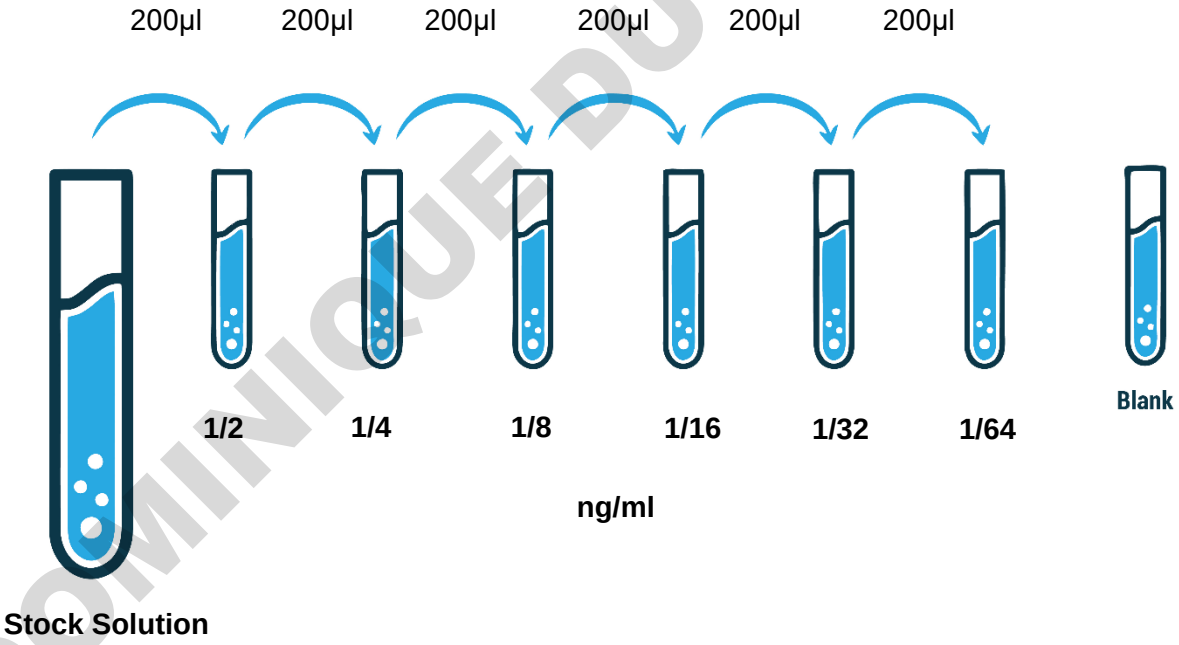
1. Reagent Preparation

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

2. Standard Dilution

Reconstitute the Standard with 1.0mL of Diluent Buffer, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 80ng/mL. Prepare 7 tubes containing 0.2mL Diluent Buffer and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Prepare a dilution series with 7 points; Label 7 Eppendorf tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively and the last EP tube with Diluent Buffer is the blank 0ng/mL.

DILUTION SERIES



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3. Detection Reagent A and Detection Reagent B

Briefly spin or centrifuge the stock Detection A and Detection B solutions before use. Dilute to the working concentrations with Diluent Buffer respectively (1:100).

4. Wash Buffer

Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).

5. TMB Substrate

Aspirate the needed dosage of the solution with sterilized tips. Do not dump the residual solution back into the vial.

8. Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 50µL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50µL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a 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 350µL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or auto-washer, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by tapping the plate onto absorbent paper. Totally wash 3 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 100µL of Detection Reagent B working solution to each well. Incubate for 1 hour at 37˚C after covering it with the Plate sealer.
4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.
5. Add 90µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15-25 minutes at 37˚C (Do not exceed 30 minutes). Protect from light. The liquid will turn blue with the addition of Substrate Solution.
6. Add 50µL of Stop Solution to each well. The liquid will turn yellow with the addition of Stop solution. Mix the liquid by tapping the side of the plate. If colour change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Remove any drops of water and fingerprints on the bottom of the plate and confirm there are no bubbles on the surface of the liquid. Run the microplate reader and conduct measurement at 450nm immediately.

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Notes

1. **Assay preparation:** Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Remaining wells should be resealed and stored at -20°C.
2. **Samples or reagents addition:** Please use the freshly prepared Standard. Carefully add samples to wells and mix gently to avoid foaming. Do not touch the well walls. 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 standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. In addition, use separated 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 in between incubation steps. Once reagents are added to the well strips, DO NOT let the strips dry at any time during the assay. Incubation time and temperature must be controlled.
4. **Washing:** The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting, and remove any drops of water or fingerprints on the bottom of the plate. Insufficient washing will result in poor precision and false 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 deep, add Stop Solution in advance to avoid excessively strong reaction which will result in an inaccurate absorbance reading.
6. **TMB Substrate** is easily contaminated. Please protect it from light.
7. The environment humidity may have an effect on the results obtained from the kit. If the humidity in your facility is less than 60%, using a humidifier is recommended.

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9. Data Analysis

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between T4 concentration in the sample and the assay signal intensity. Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log or semi-log graph paper, with the log of T4 concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points, or it can be determined by regression analysis. Using plotting software, (for instance, curve expert 1.30), is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

10. Typical Data & Standard Curve

Detection Range

1.25-80ng/mL. The standard curve concentrations used for the ELISA's were 80ng/mL, 40ng/mL, 20ng/mL, 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL and 0ng/mL.

Sensitivity

The minimum detectable dose of T4 is typically less than 0.63ng/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 adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay has high sensitivity and excellent specificity for detection of T4. No significant cross-reactivity or interference between T4 and analogs was observed.

Note: Limited by current skills and knowledge, it is impossible to perform all possible cross-reactivity detection tests between T4 and all analogs, therefore, cross reactivity may still exist.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level T4 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level T4 were tested on 3 different plates, 8 replicates in each plate.

- **CV (%) =** SD/mean X 100
- **Intra-Assay:** CV<10%
- **Inter-Assay:** CV<12%

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Stability

The stability of this ELISA Kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage conditions.

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end**.**

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Notes

1. Limited by the current conditions and scientific technology, it is impossible to conduct comprehensive identification and analysis tests on the raw materials provided by suppliers. As a result, it is possible there are some qualitative and/or technical risks.
2. The final experimental results will be closely related to the validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available to obtain accurate results.
3. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the instruction manual included in your kit. Electronic ones on our website are for reference only.
4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
5. Protect all reagents from strong light during storage and incubation. All bottle caps of reagents should be closed tightly to prevent evaporation of liquids and contamination by microorganisms.
6. There may be a foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.
7. Incorrect procedures during reagent preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment.
8. Even the same experimenter may get different results from two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before the general assay for each batch is recommended.
9. Each kit has undergone several rigorous quality control tests. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipment. Intra-assay variance among kits from different batches could arise from the above factors as well.
10. The standard in this kit, as well as the antigens used in antibody preparation are typically recombinant proteins. Differently expressed sequences, expression systems, and/or purification methods can be used in the preparation of recombinant proteins. There is also the possibility of differences in the screening technique of antibodies and antibody pairs in our kits. As a result, we cannot guarantee that our kit will be able to detect recombinant proteins produced by other companies. We do NOT recommend using Assay Genie ELISA kits for the detection of other recombinant proteins.
11. Validity period: 12 months.
12. The instruction manual also works with the 48T kit, but all reagents in the 48T kit are reduced by half.

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Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

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