Thyroxine (T4) ELISA Kit

to determine Thyroxine in Serum, Blood Plasma, Saliva, Urine, Tissue Liquid Samples or related Biological Solutions.

INSTRUCTION MANUAL

FOR ELISA KIT No: LT7000TEKKBA

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INTENDED USE

This kit is used to determine the Thyroxine (T4) in the sample of serum, blood plasma, saliva, urine, tissue liquid samples or related biological solutions. For *in vitro* use only.

MANUAL VERSION 1.02

BACKGROUND

T4 is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T4 is decreased in hypothyroid patients and is increased in hyperthyroid patients. 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 between 14:1 and 20:1. 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.

PRINCIPLE

T4 ELISA Kit employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to T4. Standards or samples are added to the appropriate microtiter plate wells with Biotin-conjugated T4. A competitive inhibition reaction is launched between T4 (Standards or samples) and Biotin-conjugated T4 with the pre-coated antibody specific for T4. The more amount of T4 in samples, the less antibody bound by Biotin-conjugated T4. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Substrate solution is added to the wells and the color develops in opposite to the amount of T4 in the sample. The color development is stopped and the intensity of the color is measured.

ASSAY RESTRICTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

MATERIALS SUPPLIED IN THIS KIT

T4 microplate: 96 well polystyrene microplates (12 strips of 8 wells) coated with the antibody specific to T4.

T4 standard: T4 in a buffered protein base; lyophilized.

Biotin Conjugated T4: liquid.

Avidin HRP: liquid.

HRP substrate A: Urea hydrogen peroxide solution.

HRP substrate B: TMB (Tetramethyl-benzidine) solution.

Stop solution: 2 mol/L sulfuric acid.

Wash buffer: PBS with 0.5% Tween-20; 20X liquid.

Plate covers.

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MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

STORAGE CONDITIONS

The unopened kit should be stored at 2-8˚C for 1 year. Immediately after use remaining reagents should be returned to cold storage at 4˚C. Recommended storage instruction for opened/reconstituted kit components are listed below.

Kit components	Storage conditions for opened components
T4 microplate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 ˚C.
T4 standard	May be stored for up to 6 months at 2-8 °C..
Biotin conjugated T4, Avidin-HRP, HRP substrate A, HRP substrate B, Stop Solution, Wash buffer	May be stored for up to 1 year at 2-8 °C.

PRECAUTIONS

1. 1. Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer. 2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8 °C in their pouch with the desiccant provided. 3. Mix all reagents before using. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25 °C).

TECHNICAL HINTS

• When mixing or reconstituting protein solutions, always avoid foaming.

• To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

• When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

• Substrate solution should remain colourless until added to the plate. Keep Substrate solution protected from light. Substrate solution should change from colorless to gradations of blue.

• Stop Solution should be added to the plate in the same order as the Substrate solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate solution.

PRECISION

Intra-assay Precision (Precision within an assay): Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%) <15%.

Inter-assay Precision (Precision between assays): Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%) <15%.

Recovery: The recovery of T4 spiked to different levels in samples throughout the range of the assay in various matrices was evaluated. The recovery ranged from 90% to 110% with an overall mean recovery of 100%

Sensitivity: The minimum detectable dose (MDD) of T4 is typically less than 10 ng/ml. The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity: T4 ELISA Kit has high sensitivity and excellent specificity for detection of T4. No significant cross-reactivity or interference between T4 and analogues was observed.

SAMPLE COLLECTION & STORAGE

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 and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20˚C. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20˚C. Avoid repeated freeze-thaw cycles. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored at -20 ˚C to avoid loss of bioactive TIMP-1. If samples are to be used within 24 hours, they may be stored at 2 to 8 ˚C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to

room temperature slowly and mixed gently.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

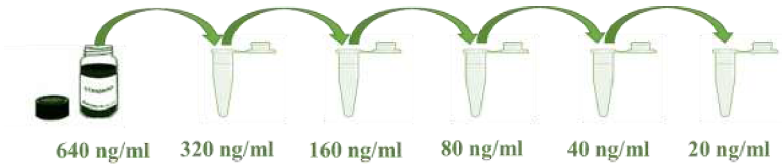
Wash buffer - Dilute with Distilled or deionized water 1:20.

Sample dilution - If your samples need to be diluted, Deionized or distilled water is used for dilution of serum/plasma samples.

ASSAY PROCEDURE

1. Dilution of standard solutions: This kit contains a standard of known concentration, which could be diluted in small tubes by the end-user by following the instruction in the table below:

320 ng/ml	Standard No.5	150µl Original Standard + 150µl Standard diluents
160 ng/ml	Standard No.5	150µl Standard No.5 + 150µl Standard diluents
80 ng/ml	Standard No.4	150µl Standard No.4 + 150µl Standard diluent
40 ng/ml	Standard No.3	150µl Standard No.3 + 150µl Standard diluent
20 ng/ml	Standard No.2	150µl Standard No.2 + 150µl Standard diluent



Prepare all reagents and working standards as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

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3. Add 50 µl of diluted standard and sample per well. Cover with the adhesive strip provided.
4. Add 50 µl of Conjugate to each well (not to Blank well). Mix well and then incubate for 60 minutes at 37 °C.

5. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash buffer (250 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or automatic washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 50 µl of avidin-HRP to each well (not to Blank well). Mix well and then incubate for 30 minutes at 37 °C.

7. Repeat the aspiration/wash process for three times as in step 5. 8. Add 50 µl of Substrate A and 50 µl of Substrate B to each well, mix well. Incubate for 15 minutes at 37 °C. Keeping the plate away from drafts and other temperature fluctuations in the dark. 9. Add 50 µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing. 10. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

PROTOCOL SUMMARY

Prepare reagents, samples and standards.

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Add 50µl samples & standards in respective wells.

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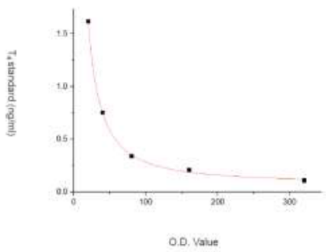
Add 50 µl of conjugate to each well and incubate for 1 hour at 37 °C.

↓

Wash the plate three times. Add 50µl of Avidin-HRP to each well (not to blank well), incubate for 30 minutes at 37 °C, and wash plate three times.

Add 50 µl HRP substrate A and 50 µl HRP substrate B solution and incubate for 10-15 minutes at 37 °C.

Add 50 µl stop solution to each well, measure O.D. at 450nm within 15 min.



Standard T4 (ng/ml)	Optical Density (450 nm)	Average
20	1.546	1.617
	1.688	
40	0.742	0.754
	0.766	
80	0.311	0.338
	0.365	
160	0.206	0.209
	0.212	
320	0.109	0.110
	0.111	

THE CHART SHOWN HERE IS FOR REFERENCE PURPOSES ONLY AND THE ACTUAL PERFORMANCE MAY VARY.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration for each standard on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the T4 concentrations versus the log of the O.D. and the best fit line can be determined by regression

analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor (for e.g. it is 5 in this case).

ASSAY RANGE : 20 ng/ml - 320 ng/ml.

PACKAGE SIZE : 96Tests.

SENSITIVITY : < 10 ng/ml.

Linearity: To assess linearity of the assay, samples containing and/or spiked with high concentrations of T4 were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

VALIDITY & STORAGE: 12 months (at 2-8°C, unopened).