

IMPORTANT NOTE

1. The T4 assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 22°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

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Manufactured by:

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Enzyme linked Immunosorbent Assay (ELISA) for Quantitative Determination of Thyroxine (T4) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ T4 Competitive ELISA test is intended for the quantitative determination of Thyroxine (T4) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

L-Thyroxine (T4) is a hormone that is synthesized and stored in the thyroid gland. Proteolytic cleavage of follicular thyroglobulin releases T4 into the bloodstream. Measurement of total T4 by immunoassay is the most reliable and convenient screening test available to determine the presence of thyroid disorders in patients. Increased levels of T4 have been found in hyper-thyroidism due to Grave's disease and Plummer's disease and in acute and subacute thyroiditis. Low levels of T4 have been associated with congenital hypothyroidism, myxedema, chronic thyroiditis (Hashimoto's disease) and with some genetic abnormalities.

PRINCIPLE OF THE ASSAY

Qualisa™ T4 Quantitative Test Kit is a Competitive based enzyme-linked immunosorbent assay. In this test, a certain amount of anti- T4 antibody is coated on microtiter wells. A measured amount of patient serum, and a constant amount of T4 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, T4 antibody in the samples and conjugated T4 compete for the limited binding sites on the anti-T4 antibody of the wells. After incubation the wells are washed and bound enzyme is detected by adding substrate. The reaction is stopped after specified time with stop solution and absorbance is determined for each well using an ELISA reader. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T4 in the sample. By reference to a series of T4 standards assayed in the same way, the concentration of T4 in the unknown sample is quantified.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

1. Coated Microwells: Microwells coated with Anti- T4 antibody.
2. T4 HRPO Conjugate Diluent.
3. T4 HRPO Enzyme Conjugate concentrate (20X).
4. TMB Substrate. Ready to use.
5. Stop Solution. Ready to use.
6. T4 Standard set of 6 Standards labeled as A to F in liquid form. Ready to use. For Standard Concentrations refer vial label.
7. Wash Buffer Concentrate (20X).

Materials required but not provided

1. Precision pipettes: 2-20µl, 10-100µl, 50-200µl, 100-1000µl
2. Disposable pipette tips
3. Distilled water
4. Disposable Gloves
5. ELISA reader
6. ELISA washer

STORAGE AND STABILITY

1. **Qualisa™ T4** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to white at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable upto one week when stored at 2-8°C.

SAMPLE COLLECTION

1. Collect Blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However Specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.

- Do not heat inactivate before use.
- Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
- Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

- Bring all reagents and specimen to room temperature before use.
- Do not pipette any material by mouth.
- Do not eat, drink or smoke in the area where testing is done.
- Use protective clothing and wear gloves when handling samples.
- Use absorbent sheet to cover the working area.
- Immediately clean up any spills with sodium hypochlorite.
- All specimens and standards should be considered potentially infectious and discarded appropriately.
- Neutralize acid containing waste before adding hypochlorite.
- Do not use kit after the expiry date.
- Do not mix components of one kit with another.
- Always use new tip for each specimen and reagent.
- Do not allow liquid from one well to mix with other wells.
- Do not let the strips dry in between the steps.

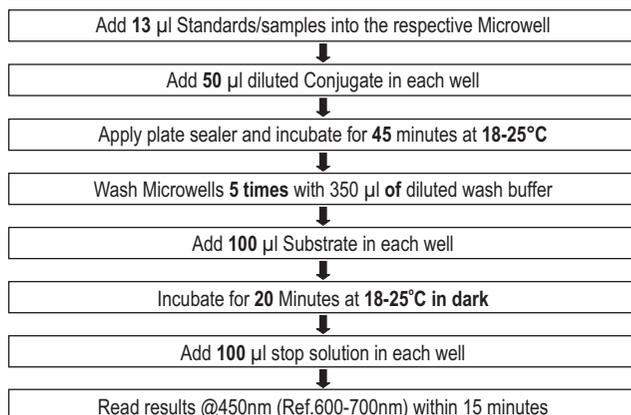
REAGENT PREPARATION

- All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
- Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- Dilute Enzyme Conjugate with Conjugate Diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.

No. of Strips	0.5	1	2	3	4	5	6	7	8	9	10	11	12
Enzyme Conjugate (µl)	12.5	25	50	75	100	125	150	175	200	225	250	275	300
Conjugate Diluent (µl)	250	500	1000	1500	2000	2500	3000	3500	4000	4500	5000	5500	6000

TEST PROCEDURE

- Secure the desired number of coated wells in the holder. Dispense 13 µl of Standards and Serums into the appropriate wells.
- Dispense 50 µl of diluted Conjugate into each well. Incubate at room temperature (18-25°C), for 45 minutes.
- Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto the absorbent paper or paper towels to remove all residual water droplets.
- Dispense 100 µl of TMB Substrate into each well. Incubate at room temperature (18-25°C) in the dark, for 20 minutes.
- Stop the reaction by adding 100 µl of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
- Read the optical density at 450/630 nm with a microtiter plate reader within 15 minutes.



CALCULATION OF RESULTS

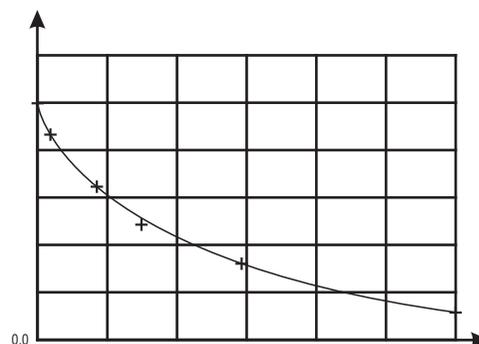
Construct a standard curve by plotting the absorbance obtained from each reference standard against its concentration in µg/dl on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of T4 in µg/dl from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical standard run with optical density reading at 450nm (ref 600-700nm) shown in the Y axis against T4 concentrations shown in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

T4 Values (µg/dl)	Absorbance
A	2.175
B	1.523
C	1.168
D	0.805
E	0.667
F	0.269



This standard curve is for the purpose of illustration only and should not be used to calculate samples. Each user should obtain his or her own standard curve and data.

Expected Ranges of values

Normal Range: 5.0 to 13.0 µg/dl.

The minimal detectable concentration of T4 by this assay is estimated to be 0.5 µg/dl.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

- Accuracy: In an internal study **Qualisa™ T4** was evaluated against commercially available licensed kit with 90 random clinical samples, & **Qualisa™ T4** has demonstrated 100% clinical correlation with the commercially available licensed kit.
- Precision: **Qualisa™ T4** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testing's	Mean Control values with Qualisa™ T4	Coefficient of Variable (CV)
Level 1	10	5.414	4.16
Level 2	10	11.257	4.83
Level 3	10	12.912	4.46

B) External Evaluation:

Qualisa™ T4 ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Qualisa™ T4** ELISA has demonstrated 100% correlation with the reference method.

*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd.).