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.

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Manufactured by:

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1119/VER-01

Qualisa™

Microwell Enzyme Immunoassay | TSH

Thyroid Stimulating Hormone

Enzyme linked Immunosorbent Assay (ELISA) for Quantitative Determination of Thyroid Stimulating Hormone in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ TSH Sandwich ELISA test is intended for the quantitative determination of Thyroid Stimulating Hormone in human serum. For InVitro Diagnostic Use only.

INTRODUCTION

TSH is secreted by the anterior lobe of the pituitary gland and induces the production and release of thyroxine and triiodothyronine from the thyroid gland. Although the concentration of TSH in the blood is extremely low, it is essential for the maintenance of normal thyroid function. TSH and the pituitary glycoproteins: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG), have identical alpha chains. The beta chain is distinct but does contain identical amino acid sequences, which can cause considerable cross-reactivity with some polyclonal TSH antisera. The use of a monoclonal antibody in this TSH EIA test eliminates this interference, which could result in falsely elevated TSH values in either menopausal or pregnant females--a population whose evaluation of thyroid status is clinically significant.

PRINCIPLE OF THE ASSAY

Qualisa™ TSH Quantitative Test Kit is a sandwich-based enzyme-linked immunosorbent assay. The test employs mouse monoclonal anti-TSH antibody for solid phase (microtiter wells) immobilization and a goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. 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 concentration of TSH is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

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

Materials required but not provided

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

STORAGE AND STABILITY

1. **Qualisa™ TSH** 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 dessicant 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.

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

PRECAUTIONS

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

REAGENT PREPARATION

1. 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.
2. Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 25 µl of Standards and Serums into the appropriate wells.
2. Dispense 50 µl of Enzyme Conjugate reagent into each well. Incubate at room temperature (18-25°C), for 45 minutes.
3. 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.
4. Dispense 100 µl of TMB Substrate into each well. Incubate at room temperature(18-25°C) in the dark, for 20 minutes.
5. 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.
6. Read the optical density at 450/630 nm with a microtiter plate reader within 15 minutes.

Add **25** µl Standards/samples into the respective Microwell

↓

Add **50** µl Enzyme conjugate in each well

↓

Apply plate sealer and incubate for **45** minutes at **18-25°C**

↓

Wash Microwells **5 times** with 350 µl **of** diluted wash buffer

↓

Add **100** µl Substrate in each well

↓

Incubate for **20** Minutes at **18-25°C in dark**

↓

Add **100** µl stop solution in each well

↓

Read results @450nm (Ref.600-700nm) within 15 minutes

CALCULATION OF RESULTS

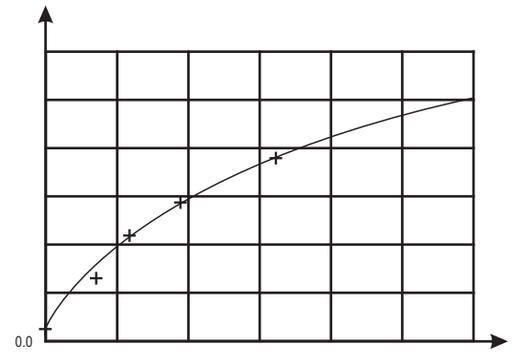
Construct a standard curve by plotting the absorbance obtained from each reference standards against its concentration in µIU/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of TSH in µIU/ml 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 TSH concentrations shown in the X axis.

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

TSH Values (µIU/ml)	Absorbance
A	0.011
B	0.052
C	0.227
D	0.426
E	0.812
F	1.438
G	2.077



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 and sensitivity

The mean TSH values based on 160 random normal adult blood samples is 1.6 (0.4-7.0) µIU/ml. The minimum detectable concentration of TSH by this assay is estimated to be 0.2 µIU/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **Qualisa™ TSH** was evaluated against commercially available licensed kit with 90 random clinical samples, & **Qualisa™ TSH** has demonstrated >98% clinical correlation with the commercially available licensed kit.

2. Precision: **Qualisa™ TSH** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testing's	Mean Control values with **Qualisa™ TSH**	Coefficient of Variable (CV)
Level 1	10	0.364	7.45
Level 2	10	5.501	6.65
Level 3	10	37.40	4.40

B) External Evaluation:

Qualisa™ TSH ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Qualisa™ TSH** ELISA has demonstrated 98% correlation with the reference method.

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

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.