TSH Quanti Microlisa

Microwell ELISA Immunoassay for the Quantitative Detection of Thyroid Stimulating Hormone (TSH) in Human Serum

1. INTRODUCTION

Thyroid Stimulating Hormone (TSH) secreted by anteriorpituitary is a glycoprotein. TSH family regulates thyroid functions. Detection of serum TSH is an important indicator of identification of thyroid functions and of research on feedback regulation mechanism of hypothalamus-pituitary-thyroid axis. Detection of serum TSH is one of the indicators of diagnostics and efficacy evaluation of hyperthyroidism and hypothyroidism, differentiate primary and secondary hypothyroidism, monitor treatment efficacy of hyperthyroidism and hypothyroidism, diagnose subclinical hyperthyroidism, screen neonatal hypothyroidism, and diagnose pituitary TSH tumor in lab.

2. INTENDED USE

TSH Quanti Microlisa is designed for in-vitro quantitative determination of Thyroid Stimulating Hormone (TSH) in human serum.

3. PRINCIPLE

TSH Quanti Microlisa is an enzyme immuno assay based on sandwich ELISA. Microwells are coated with anti-TSH antibodies. Sample is added to the microwell followed by addition of enzyme conjugate (anti-TSH labelled with HRP). Binding of TSH is detected by enzyme conjugate. Incubation is followed by a washing step to remove unbound components. The color reaction is started by addition of substrate and stopped after a defined time. The color intensity is directly proportional to the concentration of TSH in the sample.

4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2016.


Manufactured By


No. of tests


Lot Number
Batch Number


Manufacturing Date


Expiry Date


Do not use if package is damaged


Keep Dry


In vitro diagnostic medical device


See Instruction for use


Temperature Limitation


Caution
See instruction for use


Catalogue Number


Keep away from sunlight

5. PACK SIZE

- 96 Tests

6. COMPONENTS IN EACH TSH Quanti Microlisa KIT

Store all components at 2-8°C when not in use. Expiry date on the kit indicates that beyound which the kit should not be used.

TSH Quanti Microlisa Strip Plates	12 Strips (96 wells) Breakway microwells coated with anti-TSH antibodies packed in a pouch with dessicant.
Enzyme Conjugate	1 Bottle (13 ml.) Ready to use Containing peroxidase conjugated to anti-TSH antibody with preservatives.
Wash Buffer Concentrate (25x)	1 Bottle (30 ml.) PBS with surfactant. Dilute 1:25 with distilled water before use.
TMB Substrate	1 Bottle (10 ml.) To be diluted with TMB diluent before use.
TMB Diluent	1 Bottle (10 ml.) Buffer solution containing H_2O_2 with preservative
Stop Solution	1 Bottle (10 ml.) Ready to use 1N sulfuric acid
Standard-1	0 μIU/ml of TSH in Human Serum containing preservatives. *RTU
Standard-2	0.5 μIU/ml of TSH in Human Serum containing preservatives. *RTU
Standard-3	2.5 μIU/ml of TSH in Human Serum containing preservatives. *RTU
Standard-4	5.0 μIU/ml of TSH in Human Serum containing preservatives. *RTU
Standard-5	10 μIU/ml of TSH in Human Serum containing preservatives. *RTU
Standard-6	20 μIU/ml of TSH in Human Serum containing preservatives. *RTU
Standard-7	40 μIU/ml of TSH in Human Serum containing preservatives. *RTU
Plate Sealers	Adhesive backed sheets for sealing microwell plate/strips.

7. STORAGE AND STABILITY

The kit should be stored at 2-8°C in the cool and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. TSH Quanti Microlisa should not be frozen and must be protected from exposure to humidity.

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- Micropipettes and microtips.
- ELISA Reader
- Distilled or deionized water
- Graduated Cylinders, for reagent dilution
- Sodium hypochlorite solution
- Paper towels or absorbent tissue
- Timer
- Microplate washer
- Incubator 37°C
- Vortex Mixer
- Disposable gloves
- Glassware

9. SPECIMEN COLLECTION & PREPARATION

1. Only human serum samples should be used for the test. While preparing serum samples, remove the serum form the clot as soon as possible to avoid hemolysis. Fresh serum samples are preferred.
2. Specimens should be free of microbial contamination and may be stored at 2-8°C for one week, or frozen at -20°C or lower. Avoid repeated freezing and thawing.
3. Use of heat inactivated, icteric hyperlipemic and hemolyzed samples should be avoided as may give erroneous results.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

TSH Quanti Microlisa test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. If a centrifuge is available, the sample should be centrifuged. (10,000 rpm for 15 min.)

(B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

11. WARNING & PRECAUTION

CAUTION: THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves is RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

12. PRECAUTIONS FOR USE

Optimal assay performance requires strict adherence to the assay procedure described in the manual.

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Bring all the reagents & samples to room temperature (20-30°C) before use.
3. Do not combine reagents from different batches, as they are optimized for individual batch to give best results.
4. Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
5. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
6. Use freshly collected, clean serum samples for assay. Try to avoid turbid, lipemic serum or plasma samples.
7. Use a separate tip for each sample and then discard it as biohazardous waste.
8. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
9. Do not allow microwells to dry once the assay has started.
10. Run standards in each assay to evaluate validity of the kit.
11. Incubation time should not vary by more than $\pm$ 2 min.
12. Prevent evaporation during sample incubation by covering the strips with strip sealer. Remove sealer before washing.
13. Distilled or deionised water must be used for wash buffer preparation.
14. Thorough washing of the wells is critical to the performance of the assay. Overflowing of reagents or washing to adjacent wells must be prevented during washing, which may lead to incorrect results due to carry over effect.
15. Take care while preparing working substrate solution as Bottle of TMB Substrate & TMB Diluent are of same size.
16. Prepare working substrate solution just 10 minutes prior to adding in the wells.
17. Use separate tips for TMB Substrate and TMB diluent.
18. Avoid strong light exposure during the assay.
19. Ensure that the microwell strips are levelled in the strip holder. Before reading, wipe the bottom of the microwell strips carefully with soft, absorbent tissue to remove any moisture.
20. In case of any doubt the run should be repeated.

13. PREPARATION OF REAGENTS

Prepare the following reagents just before or during assay procedure. Reagents and samples should be at room temperature (20-30°C) before beginning the assay. All containers used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or deionized water. Pre-warm the incubator at 37°C.

- **TSH Quanti Microlisa strips :**

Bring foil pack to room temperature (20-30°C) before opening to prevent condensation on the microwell strips.

a. Break-off the required number of strips needed for the assay and place in the well holder. Take the strip holder with the required number of strips, taking into account that seven standards should be included in each run.

b. Unused wells should be stored at 2-8°C, with dessicant in aluminium pouch with clamp & rod. Microwells are stable for 30 days at 2-8°C from the date of opening of sealed pouch, when stored with desicant along with clamp & rod.

Caution: Handle microwell strip with care. Do not touch the bottom exterior surface of the wells.

- **Preparation of Wash Buffer:**

a) Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals dissolve.

b) Prepare at least 50 ml (2 ml concentrated buffer with 48 ml water) of buffer for each strip used. Mix well before use.

c) Mix 30 ml of 25x wash buffer concentrate with 720 ml. of distilled or deionized water. **Wash buffer is stable for 2 months when stored at 2-8°C.**

- **Preparation of working substrate solution :**

Mix TMB substrate and TMB Diluent in 1:1 ratio to prepare working substrate.

Do not store working substrate. Prepare a fresh dilution for each assay in a clean plastic/glass vessel. Determine the quantity of working substrate solution to be prepared from table. Mix solution thoroughly before use.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
TMB Diluent (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0

Discard unused working substrate solution. A deep blue color present in the substrate solution indicates that the solution has been contaminated and must be discarded.

14. PROCEDURAL NOTES:

1. Material should not be used after the expiry date shown on the labels. Components and test specimen should be at room temperature (20-25°C) before testing begins. Return the reagents to 2-8°C after use.
2. All reagents must be mixed well before use.
3. To avoid contamination, do not touch the top or bottom of strips or edge of wells.
4. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
5. Prevent evaporation during sample incubation by covering the strips with sealer; remove sealer before washing.
6. Routine maintenance of wash system is strongly recommended to prevent carry over from highly reactive specimens to non reactive specimens.

15. TEST PROCEDURE

The instructions of the procedure must be strictly followed.

The sequence of the test procedure must be carefully followed. Arrange the standards in a horizontal or vertical configuration. Configuration is dependent upon reader software. **It is recomended to perform all seven standards and samples to run in duplicate.**

(i) Fit the stripholder with the required number of TSH Quanti Microlisa coated microwell strips.

(ii) Add 50 μl of each standards and samples in respective wells. Use a separate tip for each sample and then discard as biohazardous waste.

(iii) Add 100 μl of enzyme conjugate to each well.

(iv) **Thoroughly mix for 20-30 seconds. It is important to have complete mixing of the solution in this step and dispense all reagents close to the bottom of the coated wells.**

(v) Cover the plate and incubate in an incubator at 37°C $\pm$ 1°C for 60 minutes.

(vi) Dilute the wash buffer concentrate with distilled water to 1:25 dilution.

(vii) At the end of incubation period, take out the plate from incubator and wash with working wash buffer.

WASHING: Washing can be performed either with WASHER or manually as follows:

(viii) Empty the wells.

(ix) Add 300-350μl of working washing solution into each well and give a soak time of 30 seconds.

(x) Empty the wells.

(xi) Wash each well 3 times in total.

(xii) After the third wash, tap dry the Microwells a few times on an absorbent tissue.

(xiii) Add 100 μl working substrate solution in each well.

(xiv) Incubate at room temperature (20-30°C) in dark for 15 mins. and do not expose to light.

(xv) Add 50 μl of stop solution to each well.

(xvi) Read the absorbance at 450 & 630 nm witin 15 minutes in ELISA reader.

16. SUMMARY OF PROCEDURE

Add Standards* & samples		50 μl
Add Enzyme Conjugate		100 μl
Cover the plate & incubate		60 mins. at 37°C
Wash		3 Cycles
Prepare TMB Substrate		No of Strips: 1 2 3 4 5 6 7 8 9 10 11 12 TMB Substrate (ml): 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 TMB Diluent (ml.): 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Add Substrate		100 μl
Incubate in dark		15 mins. at Room Temp.
Add Stop Solution		50 μl
Read Results		In ELISA Reader at 450 nm and 630 nm

*(Ready to use)

17. CALCULATION OF RESULTS

1. Calculate the mean absorbance values for each set of standards and samples.
2. Construct a best fit curve by plotting the absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the absorbance value for each sample determine the corresponding concentration from the best fit curve.
4. Automated Method : The results have been calculated automatically using a point to point curve fit which is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the sample can be read directly from the best fit curve. Sample with concentrations higher than that of the highest standard have to be futher diluted 1:5 or 1:10 with Standard-1 or reported as $>$ 40 μIU/ml. For the calculation of the concentration, this dilution factor has to be taken into account.
6. For subsequent run, once master curve has been established in an ELISA Reader, calculate the results with stored master curve and absorbance of 3 standards with necessary data analytics.

Important Note: QC data sheet is batch specific and can be downloaded from company web site; www.jmitra.co.in

18. EXPECTED VALUES

Each laboratory should establish its own range of normal value. The values given below are only indicative.

Distribution of normal values ranges from 0.25 μIU/ml to 5.0 μIU/ml.

19. PERFORMANCE CHARACTERISTICS

Precision :

Intra-Assay: Within precision have been determined by testing 10 replicates of 3 different samples with TSH concentration (low, medium and high value respectively) on the same lot on same day. The C.V (%) is $<$ 10%.

Inter-Assay: Between precision have been determined by testing 10 replicates of 3 different samples with TSH concentration (low, medium and high value respectively) in 10 different run at different time interval. The C.V (%) is $<$15%.

Accuracy: The accuracy of TSH Quanti Microlisa was detected with clinical specimen and compared with reference immunoassay test. The co-relation co-efficient is $\geq$0.984.

Specificity:

There was no significant interference with the TSH measurement observed when other biomolecules; LH (300 mIU/ml, FSH (200 mIIU/m and hCG (2,00,000 mIU/ml) were added to the test specimen with much higher level in normal blood.

Analytical Sensitivity :

The sensitivity is defined as being the lowest detectable concentration different from zero with a probability of 95%. The sensitivity of the TSH Quanti Microlisa kit is 0.03 μIU/ml.

Linear Range :

TSH Quanti Microlisa is linear between 0.03 μIU/ml to 40 μIU/ml.

20. LIMITATION OF THE TEST

1. Any improper handing of samples or modification of this test might influence the results.
2. Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give erroneous results.
3. No hook effect was observed in this test
4. No substances (drugs) are known to us, which have an influence to the measurement of TSH in a sample.

21. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application thereof.

22. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Standards curve out of validation limit	a) Incorrect temperature timing or pipetting	Check procedure & repeat assay
	b) Improper preparation of reagents, error of dilution, improper mixing of reagents.	Check procedure & repeat assay
	c) Cross contamination of standards/ reagents	Pipette carefully and do not interchange caps. use separate tip for standards / reagents.
	d) Used components from different lots.	Do not use components from different lots as they are adjusted for each batch released.
	e) Expired Reagents	Check the kit expiry date. Use the kit with-in shelf life
	f) Use of non calibrated micropipette and/or ELISA Reader	Calibrate micropipette and ELISA Reader at defined interval.
2. No colour developed at the end of assay	a) Any one reagent has been added in wrong sequence.	Follow the procedure meticulously & repeat assay.
	b) Inactivated conjugate, improper storage	Check storage of enzyme conjugate and it should be free of any contamination.
	c) Microplate inactivated, due to improper storage	Keep unused strips in aluminium poly pouch with the dessicant pouch inside and proerly closed with clamp & rod.
	d) Inactivated substrate, improper storage or preparation	Use freshly prepared substrate solution. Recheck procedure, repeat assay.
	e) Omission of any step in test procedure	Follow the procedure meticulously & repeat assay.
	f) Incorrect temperature timing or pipetting	Check procedure & repeat assay.
	g) Improper preparation of reagents, error of dilution, improper mixing of reagents.	Check procedure & repeat assay.
3. Too much colour in all wells of the plate (high background)	a) Contaminated substrate	Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container.
	b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc.	Check the container and quality of water used for dilution. Use of glass distilled water is preferred.
	c) Over incubation of substrate and delay in addition of stop solution.	Follow the procedure meticulously.
	d) Insufficient washing. i) Washing not consistent ii) Filling volume not sufficient. iii) Insufficient no. of wash cycles. iv) Contaminated wash device	Check wash device, fill the well close to the top. After washing, blot the microwells on absorbent tissue. Follow wash protocol meticulously
	e) Use of wash solution from other manufacturer.	Use only TSH Quanti Microlisa wash solution.
	f) Working substrate not protected from light	Incubate the plate in dark after addition of substrate.
4. Poor reproducibility	a) Washing problems. b) Uncalibrated pipettes or tips not well fitted, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	c) Reagent & sera not at room temperature or not well mixed before use.	Equilibrate reagents to room temperature and mix thoroughly before use
	d) Too long time for addition of calibrators, samples or reagents, inconsistency in time intervals.	Develop consistent and uniform technique.
	e) Interference in optical pathway due to Air bubbles.	Clean or dry the bottom of microwells, check for bubbles and repeat the readings.
5. High O.D for Standards & Samples	Beside 3a, b, c, d, e, incorrect interpretation and calculation of final results.	Check the calculation part given in the instruction manual and correctly interpret.
6. Low O.D for Standards and samples	a) Inadequate addition of standards/ substrate/conjugate solution	Follow the test procedure meticulously & repeat assay.
	b) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.
	d) Uncalibrated pipettes, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	e) Deterioration of Enzyme conjugate	Check storage of Enzyme conjugate. It shall be stored at 2-8°C.
	f) Stop solution is added before 15 minutes. Reaction terminated before 15 minutes.	Follow the test procedure meticulously.
	g) O.D. taken at incorrect wavelength.	Read O.D. values at 450 nm and 630 nm.

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in vitro diagnostic Reagent, not for medicinal use

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