T3 Quanti Microlisa

Microwell ELISA Immunoassay for the Quantitative Detection of triidothyronin (T3) in Human Serum/Plasma

1. INTRODUCTION

Triiodothyronin (T3) is a thyroid hormone with modecular weight of 651 dalton. T3 affects almost every physiological funtion of body including growth & development, metabolism, body temperature & heart rate. T3 circulate in blood as an equilibrium mixture of free & protein bound harmone in blood. T3 bound to thyroxine-binding globulin (TBG) & thyronine binding pre-albumin (TBPA). T3 is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism.

2. INTENDED USE

T3 Quanti Microlisa is designed for in-vitro quantitative determination of Total Triiodothyronin (T3) in human serum or plasma.

3. PRINCIPLE

T3 Quanti Microlisa is an enzyme immuno assay based on competitive ELISA. Microwells are coated with anti-triiodothyronin antibodies. Sample is added to the microwell followed by addition of enzyme conjugate (T3 labelled with HRPO). Binding of T3 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 inversally proportional to the concentration of T3 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 British and European Standard EN ISO 15223-1:2016.

	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution See instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight

5. PACK SIZE

- 96 Tests

6. COMPONENTS IN EACH T3 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.

T3 Quanti Microlisa Strip Plates	12 Strips (96 wells) Breakway microwells coated with anti-triiodothyronin antibodies packed in a pouch with dessicant.
Enzyme Conjugate Concentrate (11x)	1 Vial (1.5 ml) Containing peroxidase conjugated to T3 with preservatives.
Conjugate Diluent	1 Bottle (15 ml) Buffer containing binding protein inhibitors.
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
Standard-1	0 ng/ml of T3 in Human Serum containing preservatives. *RTU
Standard-2	0.5 ng/ml of T3 in Human Serum containing preservatives. *RTU
Standard-3	1.0 ng/ml of T3 in Human Serum containing preservatives. *RTU
Standard-4	2.5 ng/ml of T3 in Human Serum containing preservatives. *RTU
Standard-5	5.0 ng/ml of T3 in Human Serum containing preservatives. *RTU
Standard-6	7.5 ng/ml of T3 in Human Serum containing preservatives. *RTU
Stop Solution	1 Bottle (10 ml) Ready to use 1N sulfuric acid
Plate Sealers	Adhesive backed sheets for sealing microwell plate/strips.

RTU - Ready to use

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. T3 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 (blood samples collected without additive or anti-coagulant) or plasma (samples containing EDTA and heparin only) 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/plasma 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

T3 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. STANDARDS & 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 a suitable disinfectant solution 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 and guidelines.
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 any suitable disinfectant.

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 optimised 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. Incubation time should not vary by more than $\pm$ 2 min.
11. Prevent evaporation during sample incubation by covering the strips with strip sealer. Remove sealer before washing.
12. Distilled or deionised water must be used for wash buffer preparation.
13. 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.
14. Take care while preparing working substrate solution and use separate tips for TMB Substrate and TMB diluent.
15. Prepare working substrate solution just 10 minutes prior to adding in the wells.
16. Avoid strong light exposure during the assay.
17. 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.
18. 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.

- **T3 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 six standards should be included in each run.

b. Unused wells should be stored at 2-8°C, with dessicant in a 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 Conjugate:

Dilute Enzyme Conjugate Concentrate 1:11 in conjugate diluent. **Do not store working conjugate.** Prepare a fresh dilution for each assay in a clean glass vessel. Determine the quantity of working conjugate solution to be prepared from the table below. 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
Enzyme Conjugate Concentrate (ml.)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2
Conjugate Diluent in (ml.)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0

- **Preparation of working substrate solution :**

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

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

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.

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 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 six standards and samples to run in duplicate.

(i) Fit the stripholder with the required number of T3 Quanti Microlisa coated microwell strips.

(ii) Prepare working enzyme conjugate as specified in preparation of reagents.

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

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

(v) Gently mix the microplate for 20-30 seconds and cover.

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

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

(viii) Wash with working wash buffer.

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

a). Empty the wells.

b). Add 300-350μl of working washing solution into each well.

c). Empty the wells.

d). Wash each well 3 times in total.

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

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

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

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

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

16. SUMMARY OF PROCEDURE

Prepare working Enzyme Conjugate		No of Strips: 1 2 3 4 5 6 7 8 9 10 11 12 Enzyme Conj. Concentrate (ml): 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 Conjugate Diluent (ml.): 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0
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 ploting 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 4 PL (4 parameter logistics) 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 (1:0.5 or 1:1 with Standard-1) or reported as >7.5 ng/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 standard 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 0.52 to 1.85 ng/ml.

19. PERFORMANCE CHARACTERISTICS

Precision :

Intra-Assay: Within precision have been determined by testing 10 replicates of 3 different samples with T3 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 T3 concentration (low, medium and high value respectively) in 10 different run at different time interval. The C.V (%) is <15%.

Accuracy: The accuracy of T3 Quanti Microlisa was detected with clinical specimen and compared with reference immunoassay test. The co-relation co-efficient > 0.982.

Specificity :

The following cross reactanats were tested in the assay by diluting them in serum matrix at various concentration (0.01 μg/ml to 100 μg/ml) and calculated the cross reactivity (%CR) at 50% binding point.

Cross reactants	**%CR**
Triiodo-L-thyroxine	100%
L-Thyroxine	0.090%
3, 5 di-iodo thyronine	0.040%
Iodotyrosine	0.022%
Tetraiodothyroacetic acid	0.015%
L-Tyrosine	0.007%
D-Tyrosine	0.007%

Analytical Sensitivity :

The sensitivity is defined as being the lowest detectable concentration different from zero with a probability of 95%. The sensitivity of the assay is 0.1 ng/ml.

Linear Range :

T3 Quanti Microlisa is linear between 0.1 ng/ml to 7.5 ng/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 T3 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	Pipette carefully and do not interchange caps. Repeat assay
	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
2. If O.D is not observed at the end of assay	a) Any one reagent has been added in wrong sequence.	Check procedure and repeat assay.
	b) Inactivated conjugate used, improper conservation	Check for contamination, recheck procedure
	c) Microplate inactivated, due to improper conservation	Keep unused strips in sealable plastic bag, very well closed with the dessicant pouch inside
	d) Inactivated substrate, improper conservation or preparation	Use freshly prepared substrate solution Recheck procedure, repeat assay
3. Too much O.D in all wells of the plate	a) Contaminated substrate Use of same container for preparing & dispensing substrate & conjugate.	Check substrate (TMB Diluent) it should be colourless.
	b) Contaminated or improper dilution of reagents.	Check for contamination, check dilutions.
	c) Contaminated washing solution (1X).	Check the container and quality of water used for dilution.
	d) Insufficient washing.	Check wash device, fill the
	i) Washing not consistent	well close to the top.
	ii) Filling volume not sufficient.	After washing, blot the
	iii) Insufficient no. of wash cycles.	microwells on absorbent tissue.
	iv) Contaminated wash device	
	e) Use of Wash Buffer from other manufacturer.	Use only T3 Quanti Microlisa Wash Buffer.
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.
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 insert and correctly interpret.
6. Low O.D for Standards and samples	a) Inadequate addition of standards substrate/conjugate solution	Recheck the test procedure and reagent volume.
	b) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.

REFERENCES

1. J Clin Invest. 1972 Aug; 51(8): 1939–1949.Direct immunoassay of triiodothyronine in human serum.
2. The Journal of Clinical Endocrinology & Metabolism, Volume 36, Issue 2, 1 February 1973, Pages 397–400, SERUM AND THYROID GLAND TRIIODOTHYRONINE IN THE HUMAN FOETUS.
3. Iranian Journal of Reproductive Medicine Vol.10. No.1. pp: 47-52, January 2012 Comparison of serum levels of Tri-iodothyronine (T3), Thyroxine (T4), and Thyroid-Stimulating Hormone (TSH) in preeclampsia and normal pregnancy.
4. Archives of Disease in Childhood, 1975, 50, 565. Serum tri-iodothyronine, thyroxine, and thyrotrophin concentrations in newborns during the first 2 days of life.
5. National Journal of Medical Research, Vol. 1, Issue 2 Oct – Dec 2011, Original article variation in thyroid hormones level among people of different age, gender and seasons, Piparia, Gujarat.

For *in vitro* diagnostic use only, not for medicinal use

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