

# VITAMIN D QUANTI MICROLISA

Microwell ELISA Immunoassay for the Quantitative Detection of 25-OH Vitamin-D (total) in Human Serum

PROBLEM	POSSIBLE CAUSE	SOLUTION
4. Poor reproducibility	ii) Filling volume not sufficient.	After washing, blot the microwells on absorbent tissue.
	iii) Insufficient no. of wash cycles.	Follow wash protocol meticulously
	iv) Contaminated wash device	
	e) Use of wash solution from other manufacturer.	Use only Vitamin D Quanti Microlisa wash solution.
	f) Working substrate not protected from light	Incubate the plate in dark after addition of substrate.
	a) Washing problems.	Check all 8 ports/ manifold for uniform flow of wash buffer. If there are blockage, clean the ports.
	b) Uncalibrated pipettes or tips not well fitted, improper pipetting/ dispensing.	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 standards, controls, 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 & sample	a) Beside 3a, b, c, d, e & f incorrect interpretation and calculation of final results	Check the calculation part given in the insert and correctly interpret.
	b) High incubator temperature, incorrect timing or pipetting	Check incubator temperature, procedure & repeat assay.
	c) Use of turbid/ lipaemic or hemolyzed sample.	Centrifuge the sample at 5000 rpm for 30 minutes and re-run the test with clear sample.
6. low O.D. for Standards and samples	a) Inadequate addition of substrate/conjugate solution.	Follow the procedure meticulously & repeat assay.
	b) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.
	c) White particles in working substrate solution.	Discard the substrate and prepare the working substrate again in fresh tube.
	d) Uncalibrated pipettes, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	e) Deterioration of anti-Vitamin D Biotin conjugate and/ or Enzyme Conjugate	Check storage of Vitamin D Biotin conjugate and 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.
	h) g) Incorrect (low) incubator temperature, timing or pipetting	Check incubator temperature, pipetting & repeat assay.
	i) Kit deterioration	Check storage of kit and it should be stored at 2-8°C.
	j) Sample deterioration due to improper storage and/ or microbial contamination.	Store the sample at 2-8°C / -20°C as recommended in the specimen collection & handling.

in vitro diagnostic Reagent, not for medicinal use

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## 1. INTRODUCTION

Vitamin D is a steroid hormone and is found in two major forms of vitamin D, vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Vitamin D2 is obtained from plant sources and only represents less than 5% of the total vitamin D of the body. Vitamin D3 is produced from a cholesterol precursor, in the skin during sun exposure to ultra-violet light. Vitamin D is involved in the intestinal absorption of calcium and the regulation of its homeostasis. In the liver, the vitamin D is hydroxylated to 25-hydroxyvitamin D (25-OH D), the major circulating metabolite of vitamin D. Vitamin D and 25-OH D enter the circulation and bind to vitamin D binding protein (VDBP). The measurement of circulating 25-OH D provides better information with respect to patients vitamin D status and allows its use in diagnosis of hypovitaminosis. Determination of 25-OH D in serum will support the diagnosis and therapy control of postmenopausal osteoporosis, rickets in children, osteomalacia, renal osteodystrophy, neonatal hypocalcemia and hyperparathyroidism, cancer and cardio-vascular disease.

Vitamin D intoxication mostly occurs during a large intake of pharmaceuticals preparations of vitamin D and may lead to hypercalcemia and nephrocalcinosis in susceptible infants.

## 2. INTENDED USE

















Vitamin D Quanti Microlisa is designed for in-vitro quantitative determination of vitamin D (total) in human serum.

## 3. PRINCIPLE

Vitamin D Quanti Microlisa is an enzyme immuno assay based on delayed competitive ELISA. First samples are incubated with assay diluent to release vitamin-D from binding protein in the sample. Vitamin D biotin conjugate is added in the microwells. During incubation endogenous 25-OH vitamin D of a sample competes with a 25-OH vitamin D3-biotin conjugate for binding of the anti-vitamin D antibodies immobilized on the plate (this delayed competition increases the sensitivity for low concentration vitamin-D samples). Incubation is followed by a washing step to remove unbound components. During a second incubation, binding of 25-OH vitamin D-Biotin is detected by enzyme conjugate (peroxidase labelled streptavidin). Washing is followed. In the third incubation color reaction is started by addition of substrate and stopped after a defined time. The color intensity is inversely proportional to the concentration of 25-OH vitamin D 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 European Standard EN ISO 15223-1:2021.

	Manufactured By		In vitro diagnostic medical device
	No. of tests		Instruction for use
	Lot Number Batch Number		Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date		Catalogue Number
	Keep away from sunlight		Do not use if package is damaged
	Contains biological Material of Human Origin		Contains biological Material of Animal Origin
	Country of Manufacture		Keep Dry

## 5. KIT PRESENTATION

- 96 Tests

## 6. KIT & ITS COMPONENTS

<b>Vitamin D Quanti Microlisa Strip Plates</b>	Breakway microwells coated with anti-vitamin-D antibodies packed in a pouch with desiccant.
<b>Assay Diluent</b>	Buffer solution containing preservative
<b>Vitamin D Biotin Conjugate</b>	Containing vitamin D3 conjugated to biotin, with preservatives. (Ready to use)
<b>Enzyme Conjugate</b>	Containing peroxidase conjugated to streptavidin, with preservatives.
<b>Wash Buffer Concentrate (25x)</b>	PBS with surfactant. Dilute 1:25 with distilled water before use.
<b>TMB Diluent</b>	Buffer solution containing H <sub>2</sub> O <sub>2</sub> with preservative
<b>TMB Substrate</b>	To be diluted with TMB diluent before use.
<b>Standard-1</b>	0 ng/ml of vitamin D in Human Serum and containing 0.1% sodium azide as preservative.
<b>Standard-2</b>	4 ng/ml of vitamin D in Human Serum and containing 0.1% sodium azide as preservative.

<b>Standard-3</b>	10 ng/ml of vitamin D in Human Serum and containing 0.1% sodium azide as preservative.
<b>Standard-4</b>	25 ng/ml of vitamin D in Human Serum and containing 0.1% sodium azide as preservative.
<b>Standard-5</b>	60 ng/ml of vitamin D in Human Serum and containing 0.1% sodium azide as preservative.
<b>Standard-6</b>	130 ng/ml of vitamin D in Human Serum and containing 0.1% sodium azide as preservative.
<b>Stop Solution</b>	Ready to use, 1N sulfuric acid.
<b>Plate Sealers</b>	Adhesive backed sheets for sealing microwell plate/strips.

## 7. STORAGE AND STABILITY

Store the kit & its components at 2-8°C. Expiry date on the kit indicates the date beyond which kit should not be used.

## 8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- Micropipettes and microtips.
- Elisa reader
- Distilled or deionized water
- Graduated Cylinders, for reagent preparation
- Disinfectant Solution
- Paper towels or absorbent tissue
- Timer
- Elisa washer
- Incubator 37°C
- Vortex Mixer
- Disposable gloves
- Disinfectant Solution

## 9. SPECIMEN COLLECTION & PREPARATION

1. Only human serum should be used for the test. While preparing serum samples, remove the serum from 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. Do not use heat inactivated samples as their use may give false results. Haemolyzed and icteric hyperlipemic samples may give erroneous results.

## 10. SPECIMEN PROCESSING

### (A) FROZEN SAMPLE

Vitamin D 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. (5000 rpm for 5 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 suitable disinfectant before disposal or by autoclaving at 121°C at 15psi for 60 min. 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. Controls and standards contain Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976.)

- 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 instruction for use.**

- Do not use kit components beyond the expiration date which is printed on the kit.
- Bring all the reagents & samples to room temperature (20-30°C) before use.
- Do not combine reagents from different batches, as they are optimised for individual batch to give best results.
- Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
- Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
- Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
- Use freshly collected, clean serum samples for assay. Try to avoid turbid, lipemic serum samples.
- Use a separate tip for each sample and then discard it as biohazardous waste.
- All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
- Do not allow microwells to dry once the assay has started.
- Run all six standards, low & high controls in each assay to evaluate validity of the kit.
- Incubation time should not vary by more than  $\pm$  2 min.
- Prevent evaporation during sample incubation by covering the strips with strip sealer. Remove sealer before washing.
- Distilled or deionised water must be used for wash buffer preparation.
- 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.
- Take care while preparing working substrate solution as Bottle of TMB Substrate & TMB Diluent are of same size.
- Prepare working substrate solution just 10 minutes prior to adding in the wells.
- Use separate tips for TMB Substrate and TMB diluent.
- Avoid strong light exposure during the assay.
- 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.
- In case of any doubt the run should be repeated.

## 13. PREPARATION OF REAGENTS

Prepare the following reagents before or during assay procedures. Reagents and samples should be at room temperature (20-30°C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8°C after use. All containers used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or deionized water. Prewarm the incubator to 37°C.

### ● Vitamin D Quanti Microlisa strips:

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

- 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 desiccant in an 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 desiccant along with clamp & rod.

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

### ● Preparation of Working Wash Buffer:

- Prepare at least 25 ml (1.0 ml concentrated buffer with 24.0 ml water) of buffer for each ELISA strip used. Mix well before use.

- Mix 20 ml of 25x wash buffer concentrate with 480 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.

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)	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	8.8	9.6
TMB Diluent (ml)	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	8.8	9.6

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

## 14. PROCEDURAL NOTES:

- Material should not be used after the expiry date shown on the labels. Components and test specimen should be at room temperature (20-30°C) before testing begins. Return the reagents to 2-8°C after use.
- All reagents must be mixed well before use.
- To avoid contamination, do not touch the top or bottom of strips or edge of wells.
- All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
- Prevent evaporation during sample incubation by covering the strips with sealer; remove sealer before washing.

## 15. TEST PROCEDURE

**The instructions of the procedure must be strictly followed.**

The sequence of the procedure must be carefully followed. Arrange the standards and controls in a horizontal or vertical configuration. Configuration is dependent upon reader software. It is recommended to include all six standards in each run along with samples, low & high control.

### 15.1 ELISA Procedure

- Secure the required no. of appropriate coated microwells for the assay.
- Add **25µl** of each **standard(1-6), controls and samples** into the respective wells.
- Add **100µl** of **Assay diluent** in each well, and thoroughly mix for 10-20 sec.
- Cover the microwell plate and Incubate at **37°C for 45 minutes.**
- Add **50µl** of **vitamin D biotin conjugate** to each well and thoroughly mix for 10-20 sec.
- Cover the microwell plate and Incubate at 37°C for 15 minutes.
- Dilute the wash buffer concentrate with distilled water to 1:25 dilution.
- Wash the well after 15 minutes.

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

- Empty the wells.
- Add 300-350µl of working washing solution into each well.
- Empty the wells.
- Wash each well **3 times** in total.
- After the third wash, tap dry the Microwells a few times on an absorbent tissue.
- Add **100µl enzyme conjugate** solution in each well.
- Cover the plate and incubate at **Room Temperature for 15 minutes.**
- Wash each well **3 times** in total. (Refer steps (vii a-e)).
- Add **100 µl working substrate** solution in each well.
- Incubate at room temperature in dark for **15 mins.** and do not expose to light.
- Add **50 µl of stop solution** to each well.

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

## 16. CALCULATION OF RESULTS

- Calculate the mean absorbance values for each set of standards, controls and samples.
- Construct a standard 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 as log (X) - logit (Y) by competitive method.
- Using the absorbance value for each sample, determine the corresponding concentration from the standard curve.
- 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.
- The concentration of the sample can be read directly from the standard curve. Sample with concentrations higher than that of the highest standard have to be further diluted (1:0.5 to 1:2) or reported as > 130 ng/ml. For the calculation of the concentration, this dilution factor has to be taken into account.
- For subsequent run, once master curve has been established in an ELISA Reader, calculate the results with stored master curve and absorbance of 3 calibrators with necessary data analytics.

**Important Note: QC data sheet is batch specific and can be downloaded from company web site; [www.jmitra.co.in](http://www.jmitra.co.in)**

## 17. 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 30 ng/ml to 100 ng/ml.

Vitamin D status	25-OH vitamin D (ng/mL)	25-OH vitamin D (nmol/L)
Deficiency	< 10	< 25
Insufficiency	10 - 29	25 - 72.5
Sufficiency	30 - 100	75 - 250
Toxicity	> 100	> 250

## 18. PERFORMANCE CHARACTERISTICS

### 18.1 Conformity with NIST Standards

The results of Vitamin D Quanti Microlisa were in conformity with NIST standards. Standard Reference Material (972a) containing level 1, 2, 3 & 4 for Vitamin D from National Institute of Standards & Technology (NIST), US Department of Commerce, USA; were checked on Vitamin D Quanti Microlisa and results were comparable and are tabulated as below:

### NIST Standard Reference Material (SRM) for Vitamin D metabolites

Levels	Certified Values in NIST SRM 972a (ng/mL)	Value Observed in Vitamin D Quanti Microlisa (ng/mL)	Co-relation
Level 1	28 + 1.1	28.6	Results with all 4 levels of standard are within defined range and found
Level 2	20.20 + 0.52	19.9	
Level 3	33.1 + 0.8	33.2	
Level 4	29.4 + 0.9	28.9	to be in co-relation.

### 18.2 Accuracy:

The accuracy of Vitamin D Quanti Microlisa was checked with clinical specimens and compared with various reference tests. The accuracy parameters are as given below:

Method	No. of Samples	Correlation Coefficient
LC-MS/MS	45	0.97
Diasorin 25-OHvitaminD total Liaison	245	0.932
Euroimmune 25-OHvitaminD total ELISA	132	0.921

### 18.3 Linearity:

Vitamin D Quanti Microlisa is linear between 2.57 to 130 ng/ml.

### 18.4 Specificity of antibodies (Cross Reactivity):

Cross-reactivity (CR%) of the Vitamin-D Quanti Microlisa was determined by diluting following analogues in serum metrics.

Analogues	CR%
25 OH Vitamin D3	100%
25 OH Vitamin D2	100%
1,25 (OH)2 vitamin D3	0.73%
Vitamin D3	0.9%
Vitamin D2	0.5%

**Interfering substance:** The potential interfering substance like Heamolysed, lipemic and high bilirubin serum samples have no influence on the ELISA results.

### 18.5 Sensitivity:

The analytical sensitivity of the Vitamin D Quanti Microlisa is 2.57 ng/mL.

### 18.6 Reproducibility:

#### 18.6.1 Intra Assay

The within assay variability is as shown below:

Sample	n	Mean (ng/mL)	CV(%)
1	10	7.1	5.2
2	10	37.2	7.7
3	10	88.6	9.8

#### 18.6.2 Inter Assay

The between assay variability is as shown below:

Sample	n	Mean (ng/mL)	CV(%)
1	10	6.92	14.8
2	10	36.3	13.2
3	10	87.9	8.5

## 19. LIMITATIONS OF THE ASSAY

- Any improper handling of samples or modification of this test might influence the results.
- Samples which show turbidity, hemolysis, hyperlipemia or contain fibrin may give erroneous results.
- No hook effect was observed in this test.
- No substances (drugs) are known to us, which have an influence to the measurement of 25 OH vitamin D 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 for use, 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 manufacturer'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 there of.

## 22. REFERENCES

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## 23. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Standards curve out of validation limit	<ol style="list-style-type: none"> <li>Incorrect temperature timing or pipetting</li> <li>Improper preparation of reagents, error of dilution, improper mixing of reagents.</li> <li>Cross contamination of standards/ reagents</li> <li>Used components from different lots.</li> <li>Expired Reagents</li> <li>Use of non calibrated micropipette and/or ELISA Reader</li> </ol>	<p>Check procedure &amp; repeat assay</p> <p>Check procedure &amp; repeat assay</p> <p>Pipette carefully and do not interchange caps. use separate tip for standards / reagents.</p> <p>Do not use components from different lots as they are adjusted for each batch released.</p> <p>Check the kit expiry date. Use the kit with-in shelf life</p> <p>Calibrate micropipette and ELISA Reader at defined interval.</p>
2. No colour developed at the end of assay	<ol style="list-style-type: none"> <li>Any one reagent has been added in wrong sequence.</li> <li>Deterioration of anti-Vitamin D Biotin conjugate and/ or Enzyme Conjugate</li> <li>Microplate inactivated, due to improper storage</li> <li>Inactivated substrate, improper storage or preparation</li> <li>Omission of any step in test procedure</li> <li>Incorrect temperature timing or pipetting</li> <li>Improper preparation of reagents, error of dilution, improper mixing of reagents.</li> <li>Kit expired</li> </ol>	<p>Follow the procedure meticulously &amp; repeat assay.</p> <p>Check storage of Vitamin D Biotin conjugate and Enzyme Conjugate. It shall be stored at 2-8°C.</p> <p>Keep unused strips in aluminium poly pouch with the dessicant pouch inside and properly closed with clamp &amp; rod.</p> <p>It should be free of any contamination. Use freshly prepared substrate solution. Recheck procedure and repeat assay.</p> <p>Follow the procedure meticulously &amp; repeat assay.</p> <p>Check procedure &amp; repeat assay</p> <p>Check procedure &amp; repeat assay</p> <p>Check storage and expiry of the kit before use and should be stored at 2-8°C.</p>
3. Too much colour in all wells of the plate (high background)	<ol style="list-style-type: none"> <li>Contaminated substrate</li> <li>Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc.</li> <li>Over incubation of substrate and delay in addition of stop solution.</li> <li>Insufficient washing.</li> <li>Washing not consistent</li> </ol>	<p>Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container.</p> <p>Check the container and quality of water used for dilution. Use of distilled water is preferred.</p> <p>Follow the procedure meticulously.</p> <p>Check wash device, fill the well close to the top.</p>