PCT Quanti Microlisa

Microwell ELISA Immunoassay for the Quantitative Detection of PCT in Human Serum/Plasma

1. INTRODUCTION

Procalcitonin (PCT) the precursor of the hormone calcitonin, is a 116 amino acid protein with a molecular mass of 13 kDa, encoded by CALC1 gene.

Under normal conditions all the PCT are formed in thyroid C cells and converted to calcitonin so that no PCT is released into circulation therefore the level of procalcitonin in the blood of healthy individuals is low (<0.05 ng/ml). But during inflammation, PCT is not converted in calcitonin and produced from cells by two mechanism; direct pathway induced by bacterial lipopolysacharide and indirect pathway induced by inflammatory mediators like IL-6, TNF-α, etc. So, the risk of systemic bacterial infection occurs when the value of procalcitonin exceeds 0.5 ng/ml.

Procalcitonin levels increase from 3 to 4 hours, peak at about 6 hours and then plateau for up to 24 hours.

2. INTENDED USE

PCT Quanti Microlisa is designed for in-vitro quantitative determination of PCT in human serum or plasma.

3. PRINCIPLE

PCT Quanti Microlisa is an enzyme immuno assay based on sandwich ELISA. Microwells are pre-coated with antibodies against human procalcitonin. Sample is added to the microwell followed by addition of enzyme conjugate (anti-PCT antibodies labelled with HRPO). Binding of PCT 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 PCT 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	*In vitro* diagnostic medical device
No. of tests	See Instruction for use
Lot Number Batch Number	Temperature Limitation
Manufacturing Date	Caution See instruction for use
Expiry Date	Catalogue Number
Do not use if package is damaged	Keep away from sunlight

5. PACK SIZE

- 96 Tests

6. COMPONENTS IN EACH PCT QUANTI MICROLISA KIT (96 TESTS)

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.

PCT Quanti Microlisa Strip Plates	12 Strips (96 wells) Breakway microwells pre-coated with anti-PCT antibodies in a pouch with dessicant.
Enzyme Conjugate	1 Vial (7 ml.) (Ready to use) Containing anti-PCT antibodies labelled with HRPO 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
Standard-1	1 Vial (1 ml): 0 ng/ml of PCT in Human Serum containing preservatives. (Ready to use)
Standard-2	1 Vial (1 ml): 0.5 ng/ml of PCT in Human Serum containing preservatives. (Ready to use)
Standard-3	1 Vial (1 ml): 1 ng/ml of PCT in Human Serum containing preservatives. (Ready to use)
Standard-4	1 Vial (1 ml): 2.5 ng/ml of PCT in Human Serum containing preservatives. (Ready to use)
Standard-5	1 Vial (1 ml): 10 ng/ml of PCT in Human Serum containing preservatives. (Ready to use)
Standard-6	1 Vial (1 ml): 25 ng/ml of PCT in Human Serum containing preservatives. (Ready to use)
Stop Solution	1 Bottle (15 ml) Ready to use 1N sulfuric acid
Plate Sealers	Adhesive backed sheets for sealing microwell plate/strips.

7. STORAGE AND STABILITY

Store the kit & its component 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 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 or plasma samples (EDTA, heparin or citrate plasma) 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.
4. Do not use samples containing sodium azide.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

PCT 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 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 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 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 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. Run all six standards in each assay to evaluate validity of the kit.
11. Incubation time should not vary by more than $\pm$ 1 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.

- **PCT 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 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 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.

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. 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:

1. 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.
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 and samples in a horizontal or vertical configuration. Configuration is dependent upon reader software. **It is recommended to include all six standards in each run.**

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

(ii) Add 50 μl of each standard and sample (serum/ plasma) in respective wells.

(iii) Add 50 μl of enzyme conjugate to each well and mix well.

(iv) **Throughly mix for 10 seconds. It is important to have complete mixing of the solution in this step.**

(v) Cover the plate and incubate in an incubator at 37°C $\pm$ 1°C for 30 minutes.

(vi) Wash the wells 3 times with working wash solution (300-350 μl). After final wash, invert and tap the plate strongly against paper towel.

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

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

(ix) Add 100 μl of stop solution to each well.

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

16. SUMMARY OF PROCEDURE

Add Standards & samples		50 μl
Add Enzyme Conjugate		50 μl
Cover the plate & incubate		30 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		100 μl
Read Results		In ELISA Reader at 450 nm and 630 nm

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 > 25 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 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.

- PCT values below 0.1 ng/ml are considered as normal.
- Values between 0.1 ng/ml to 0.5 ng/ml are considered as of local infection.
- Values between 0.5 ng/ml to 2 ng/ml are considered as of sepsis.
- Values between 2 ng/ml to 10 ng/ml are considred as of severe sepsis.
- Values above 10 ng/ml are considred as of septic shock.

19. PERFORMANCE CHARACTERISTICS

Precision

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

Linear Range :

PCT Quanti Microlisa is linear between 0.010 ng/ml to 25 ng/ml.

Analytical Sensitivity

The sensitivity is defined as being the lowest detectable concentration different from zero with a probability of 95%. The sensitivity of the PCT Quanti Microlisa kit is 0.010 ng/ml.

Specificity

There was no significant interference with the PCT measurement observed when other biomolecules; CRP (200 ng/ml) and Bilirubin (20 ng/ml) were added to the test specimen with much higher level in normal blood.

Accuracy: The accuracy of PCT Quanti Microlisa was detected with 25 clinical specimen and compared with reference immunoassay test. The co-relation co-efficient is $\geq$0.996.

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 PCT 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 there of.

22. REFERENCES

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23. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. standardsout 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
	f) Use of non calibrated micropipette and/or ELISA Reader	Calibrate micropipette and ELISA Reader at defined interval.
2. If absorbance is not observed at the end of assay	a) Any one reagent has been added in wrong sequence.	Check procedure and repeat assay.
	b) Conjugate deterioration due to improper storage	Check for contamination, recheck procedure
	c) Microplate deterioration, due to improper storage	Keep unused strips in sealable plastic bag, very well closed with the dessicant pouch inside
	d) Deterioration substrate, improper storage or preparation	Use freshly prepared substrate solution Recheck procedure, repeat assay
3. Too much absorbance in all wells of the plate	a) Contaminated substrate use of same container for preparing & dispensing substrate & conjugate.	Check substrate (substrate 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	
	f) Use of wash solution from other manufacturer.	Use only PCT Quanti Microlisa wash solution.
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 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 instruction manual and correctly interpret.
6. Low O.D. for Samples & Standards	a) Inadequate addition of 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.
7. More than one plate used in single run	Affecting dose curve	Repeat dose curve in second plate or repeat minimum 3 standards and adjust with master curve.
8. Non-calibrated ELISA Reader	Wrong Result	Calibrate at specified intervals.
9. Inadequate equipment maintenance	Wrong result	Preventive maintenance
10. Sample other than serum/ plasma	False result	Specimen of suitable sample types in IFU.
11. Incorrect reagent Storage	Sensitivity & Specificity issues	Store at 2-8ºC.
12. Standard are not run in Subsequent run	Problem in dose curve calculation and result interpretation	Always run standards in subsequent run.
13. Confusion in units	Problem in measurement	Unit displayed and printed with all results.
14. Time deviation during kinetic reaction of TMB Substrate	Wrong interpretation of results	The Substrate and Stop solution should be added in the same sequence to eliminate any time deviation during reaction
15. Variation in incubator temp.	Variation in absorbance	Incubator temp. should be checked before use.
16. Components of kits are not at R.T.	Improper results	The components present in kits should come at R.T. before use.

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

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