hs-CRP Quanti Microlisa

Microwell ELISA Immunoassay for the Quantitative Detection of CRP (C-Reactive Protein) in Human Serum/Plasma

1. INTRODUCTION

C-Reactive Protein has traditionally been used to diagnose and monitor acute inflammation. It was named as such for its ability to bind and precipitate the C-polysacchride of pneumococcus. It is an alpha globulin (MW 110-140 kD). CRP is synthesized in the liver and is normally present as a trace constituent of serum or plasma at levels less than 0.3 mg/dl. It has numerous physiological functions similar to those of immunoglobulins and acts as a host defense mechanism.

CRP is one of the acute-phase proteins, the serum or plasma levels of which rise during general, nonspecific response to a wide variety of diseases. This include infections by gram-positive and gram-negative organisms, acute phase of rheumatoid arthritis, abdominal abscesses, and inflammation of the bile duct. CRP may also be found in patients with Guillain-Barre syndrome and multiple sclerosis, certain viral infections, tuberculosis, acute infectious hepatitis, many other necrotic and inflammatory diseases, burned patients and after surgical trauma.

Although the detection of elevated levels of CRP in the serum is not specific for any particular disease, it is a useful indicator of inflammatory processes. CRP levels rise in serum or plasma within 24 to 48 hours following acute tissue damage, reach a peak during the acute stage (approximately 1000x consecutive level) and decrease with the resolution of inflammation or trauma. The concentration increase of CRP in human serum or plasma may last for several days before decreasing to normal levels.

The detection of CRP is a more reliable and sensitive indicator of the inflammatory process than the erythrocyte sedimentation rate. which may also be influenced by physiological changes not associated with an inflammatory process.

Since, elevated levels of CRP are always associated with pathological changes, the CRP assays provide useful information for the diagnosis and therapeutic monitoring of inflammatory processes and associated diseases. Measurement of CRP by high sensitive CRP assays adds to the predictive value of other cardiac markers like Myoglobin, CKMB, hs-CRP, Troponin-I and T to access the risk of cardiovascular and peripheral vascular disease.

2. INTENDED USE

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

3. PRINCIPLE

hs-CRP Quanti Microlisa is high-sensitivity C-reactive protein enzyme immuno assay based on sandwich ELISA. Microwells are coated with streptavidin. Sample is added to the microwell followed by addition of enzyme conjugate (anti-CRP labelled with HRPO and biotin). Binding of CRP 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 CRP 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 hs-CRP QUANTI MICROLISA KIT (96 TESTS)

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

hs-CRP Quanti Microlisa Strip	4 Pouch (24 wells) Breakway microwells coated with streptavidin packed in a pouch with dessicant.
Enzyme Conjugate	1 Vial (13 ml.) (Ready to use) Containing biotin labelled monoclonal IgG antibody and peroxidase conjugated to anti-CRP antibody with preservatives.
Wash Buffer Concentrate (25x)	1 Bottle (30 ml.) PBS with surfactant. Dilute 1:25 with distilled water before use.
Sample Diluent	1 Bottle (50 ml.) Buffer containing protein stabilizers and antimicrobial agents as preservative.
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 (0.5 ml): 0 mg/L of hs-CRP in Human Serum with preservative. *RTU
Standard-2	1 Vial (0.5 ml) : 0.005 mg/L of hs-CRP in Human Serum with preservative. *RTU
Standard-3	1 Vial (0.5 ml) : 0.010 mg/L of hs-CRP in Human Serum with preservative. *RTU
Standard-4	1 Vial (0.5 ml) : 0.025 mg/L of hs-CRP in Human Serum with preservative. *RTU
Standard-5	1 Vial (0.5 ml) : 0.050 mg/L of hs-CRP in Human Serum with preservative. *RTU
Standard-6	1 Vial (0.5 ml) : 0.100 mg/L of hs-CRP in Human Serum with preservative. *RTU
Stop Solution	1 Bottle (15 ml) Ready to use 1N sulfuric acid
Microwell Frame	1 No.
Plate Sealers	Adhesive backed sheets for sealing microwell plate/strips.

RTU - Ready to use

7. STORAGE AND STABILITY

The kit should be stored at 2-8ºC 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. hs-CRP 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
- Paper towels or absorbent tissue
- Glassware
- Timer
- Microplate washer
- Incubator 37ºC
- Vortex Mixer

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^{0}C for one week, or frozen at -20^{0}C or lower. Avoid repeated freezing and thawing.
3. Use of heat inactivated, icteric hyperlipemic and hemolyzed and Icteric hyperlipemic samples should be avoided as may give erroneous results.
4. Do not use samples containing sodium azide.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

Hs-CRP 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 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.

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^{o}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^{o}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.

- **hs-CRP Quanti Microlisa strips :**

Bring foil pack to room temperature (20-30^{o}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^{o}C, with dessicant in an aluminium pouch 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^{0}C until all crystals dissolve.

b) 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.

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^{0}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.

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^{0}C) before testing begins. Return the reagents to 2-8^{0}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.SAMPLE PREPERATION:

TUBE DILUTION: Mark the tubes carefully for the proper identification of the samples. Dilute the serum samples to be tested, with sample diluent 1:101 in separate tubes (500 μl. diluent + 5 μl serum samples) do not dilute standards.

16. 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 recomended to perform all six standards and samples to run in duplicate.

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

(ii) Add 10 μl of each standards (Ready to use) in respective wells.

(iii) Add 10 μl of diluted sample into the respective wells.

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

(v) **Throughly mix for 10 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.**

(vi) Cover the plate and incubate in an incubator at 37^{o}C $\pm$ 1^{o}C for 45 minutes.

(vii) Dilute the wash buffer concentrate with distilled water to 1:20 dilution.

(viii) 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:

(ix) Empty the wells.

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

(xi) Empty the wells.

(xii) Wash each well 4 times in total.

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

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

(xv) Incubate at room temperature (20-30^{o}C) in dark for 15 mins. and do not expose to light.

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

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

17. CALCULATION OF RESULTS

1. Calculate the mean absorbance values for each set of standards and samples.
2. Construct a standard curve by ploting the absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration in mg/L on the horizontal (X) axis.
3. Using the absorbance value for each sample determine the corresponding concentration of CRP (mg/L) from the bestfit curve.
4. Automated Method : The results have been calculated automatically using a point to point curve fit which is the prefered method. Other data reduction functions may give slightly different results.
5. The obtained value of patients sample should be multiplied by the dilution factor of 100 to obtained CRP concentration (mg/L).
6. Absorance of sample higher than that of the highest standard (0.100 mg/L) absorbance have to be futher 50 fold diluted after the initial 100 fold dilution (total 1:5000 dilution and the final CRP values should be multiplied by 5000 to obtained CRP results in mg/L.
7. Patient samples with CRP concentration less than 0.100 mg/L should reported <0.100 mg/L CRP.

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.

For people the diagnosis of inflammatory disease and cardiovascular disease assessment cut-offs have been recomended as follows:

Concetration	Clinical Reference
<1.0 mg/L	Low CVD risk (No Inflmmation Situation)
1.0 - .3.0 mg/L	Moderate CVD risk (No Inflammation Situation)
>3.0 mg/L	High CVD risk (No Inflammation Situation)
>10 mg/L	There may be other infections (bacterial infections or viral infections)
10 - 20 mg/L	Generally indicates viral infections or mild bacterial infection
20 - 50 mg/L	Generally indicates moderate bacterial infection
>50 mg/L	Generally indicates serious bacterial infection

19. PERFORMANCE CHARACTERISTICS

Precision :

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

Accuracy: The accuracy of hs-CRP 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 CRP measurement observed when other biomolecules; Triglycerides, human-IgG, Lipids & Billirubin 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 hs-CRP Quanti Microlisa kit is 0.100 mg/L.

Linear Range :

hs-CRP Quanti Microlisa is linear between 0.100 mg/L to 10 mg/L.

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 CK-MB 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. REFERENCE

1. Pearson TA, et al. Circulation. 2003; 107:499-511
2. Ridker PM. Circulation. 2003; 107:363-369. http://circ.ahajournals.org/cgi/content/full/107/3/363
3. Dedobbeleer C, et al. Acta Cardiol. 2004;59(3):291-296. http://www.ncbi.nim.nih.gov/pubmed/15255461?

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
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) Inactivated conjugate, wrong dilution 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 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 Quanti hs-CRP 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. None calibrated ELISA Reader	Wrong Result	Specified intervalsof calibration
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	Storage requirement mentioned in IFU & Box.
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 during reaction	The Substrate and Stop solution should be added in the same sequence to eliminate any time deviation
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.

R-01

VER-01

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

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