UMA CO., LTD.

2-19-6 Yokosuka

Matsudo, Chiba, Japan

MEASURE CRP

Reagent for determination of C-reactive protein

Latex turbidity Method

2 - 8°C	**IVD** *In vitro* Diagnostics	**QUALITY MANAGEMENT SYSTEM (BY TUV)**
DO NOT freeze	12 months/block from light	**ISO 13485:2016**

1. PURPOSE OF USE

Providing a quantitative in vitro assay for the C-reactive protein (CRP) concentration in serum or plasma.

2. GENERAL INSTRUCTION

a. For in vitro diagnostics use only.

b. Diagnosis should be made in a comprehensive manner, in accordance with other related test results and clinical symptoms by the doctor in attendance.

c. For guaranteed results, usage of this product must comply with the instruction in this manual.

d. If you use automatic analyzers, follow their instructions carefully.

SUMMARY

Atherosclerosis is a chronic inflammatory condition that is triggered by initial oxidative modification of lipoproteins within the arterial space. C-reactive protein (CRP) is an inflammatory protein biomarker that is thought to predict cardiovascular disease risk. Although relatively few clinical studies have examined inflammatory status in response to plant sterols consumption, the majority of this work has failed to see an effect of plant sterol therapy on the concentration of CRP and other acute markers of systemic inflammation including tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6).

3. MATERIALS REQUIRED BUT NOT INCLUDED

- Saline 0.9 % and high grade purified water

- Micropipet and other basic laboratory equipment.

- CRP Calibrator Set and CRP Control Set

4. REAGENT COMPOSITION & PREPARATION

- Reagent R-1: Saline Buffer

Reagent R-1 is ready for use

- Reagent R-2: anti-human CRP rabbit antibody- sensitized latex

Reagent R-2 is ready for use

- Once open, Reagent stored on board the instrument is stable for 30 days with Hitachi 7180 Analyzers.

- Applicable to various automated analyzers.

- Calibrator (separately sold): Ready for use

- Controls Low & High (separately sold): Ready for use.

5. SAMPLE PREPARATION & STORAGE

- Serum: Wait until the sample is completely coagulated. Take the supernatant to use as specimens.

- Plasma: Treat blood sample by anticoagulant (Li-heparin and K2-EDTA); leave it to stand for 3 hours or centrifuge at 2000 rpm for 2 minutes; take the plasma layer (supernatant) and use as specimens.

- Analyze samples soon after collection. In case, it could not be analyzed soon, store sample 2 – 8°C and analyze within 3 days.

- Stability:

- 8 hours at 15 - 25°C
- 3 days at 2 - 8°C
- 6 months at < -20°C

- See interferences section for details about possible sample interferences.

6. MEASUREMENT PRINCIPLE

When a latex reagent is made to react with a specimen, the CRP in the specimen and anti-human CRP rabbit antibody-sensitized latex in the latex reagent produce a specific antigen-antibody reaction, resulting in turbidity.

As the degree of turbidity is in proportion to the concentration of CRP in a specimen, the turbidity is measured optically to determine the concentration CRP.

7. ASSAY PROCEDURE

This product is compatible with various types of clinical analyzer. An example of the assay procedure is indicated below.

Sample	+	Reagent 1	$\xrightarrow[280s]{37^\circ C}$	Reagent 2
2.4µL		120µL		120µL
$\xrightarrow[55s]{37^\circ C}$		Meas. Abs I	$\xrightarrow[265s]{37^\circ C}$	Meas. Abs II
		(570/800nm)		(570/800nm)

$\longrightarrow$ ΔAbs at 570/800nm $\longrightarrow$ Concentration CRP

Perform the assay according to the instructions for operating the automated analyzer Hitachi models. Refer to the **13. INFORMATION FOR AUTOANALYZERS** for the details of the assay method. Contact **HUMA MEDICAL CO., LTD.** for information about the parameters for other automated analyzers.

8. CALCULATION & UNIT CONVERSION

Calculation

- Calculate ΔAbs of specimen & standards vs blank

- Plot a calibration curve CRP = f(ΔAbs)

- Calculate CRP in specimen using the curve

(doing same procedure for Controls)

Unit conversion

mg/dL x 10 = mg/L

9. PERFORMANCE & CORRELATION TEST

a. Measuring range

- The assay is linear within an CRP concentration range in serum/plasma of 0.1 - 320 mg/L.

- If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

b. Detection Limit

Limit of Blank (LoB)	=	0.0 mg/L
Limit of Detection (LoD)	=	0.1 mg/L
Limit of Quantitation (LoQ)	=	0.1 mg/L

The LoB, LoD and LoQ were determined in accordance with CLSI EP17-A2 requirements.

The LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoB corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

The LoD is determined based on the LoB and standard deviation of low concentration samples. The LoD corresponds to the lowest analyte concentration which can be detected (value above the LoB with a probability of 95%).

The LoQ is the lowest analyte concentration that can be reproducibly measured with a total error of 20%. It has been determined using low concentration samples.

c. Performance

- Sensitivity: Absorbance of a sterile saline sample is less than 0.015. Absorbance of a 10 mg/L CRP sample is about 0.02 - 0.12.

- Accuracy: When measuring a control sample, the result is within ±10% of assigned value.

d. Precision (on Biolis 30i / SK300)

Representative performance data on the analyzers are given below.

Results obtained in individual laboratories may differ.

Precision was determined using controls followed the CLSI Approved Guidline EP5-A2 with repeatability, reproducibility and total precison (1 aliquot per run, 2 run per day, 20 days).The following results were obtained.

Criterion: CV of Repeatability (aka. Within-run precision) is less than 2% and Total Precision is less than 5%.

Repeatability	*Mean mg/L*	*SD mg/L*	*CV %*
CRP Control Low	11.3	0.12	1.04
CRP Control High	27.6	0.20	0.71

Reproducibility	*Mean mg/L*	*SD mg/L*	*CV %*
CRP Control Low	11.3	0.31	2.78
CRP Control High	27.6	0.67	2.44

Total precision	*Mean mg/L*	*SD mg/L*	*CV %*
CRP Control Low	11.3	0.32	2.87
CRP Control High	27.6	0.69	2.49

Reference Material for Calibration

IRMM ERM-DA474/IFCC

10. EXPECTED VALUES

Less than 3 mg/L

Reference range should be established at each facility and judgement should be based on measurement results in a comprehensive manner together with clinical symptoms and other measurement results.

11. INTERFERENCES

- Icterus: No significant interference of conjugated bilirubin concentration up to 20 mg/dL and free bilirubin concentration up to 20 mg/dL

- Hemolysis: No significant interference of hemoglobin concentration up to 500 mg/dL

- Lipemia (Intralipid): No significant interference triglycerides concentration up to 3000 FTU

- Ascorbic Acid: No significant interference of ascorbic acid concentration up to 50 mg/dL

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Please use another methods if the result is affected by any factors

12. HANDLING, USAGE & DISPOSAL

Handling

1. Specimen can be potentially positive for infectious agents including hepatitis B virus and HIV. Wear glove and goggle when needed.

2. In case reagents got into skin, eye or mouth by mistake, wash it immediately with plenty of water and consult the doctor if needed.

3. If reagents are spilled, dilute with water and wipe it out. If specimen is spilled, spray 80% of alcohol over the specimen and wipe it out.

Usage

1. Store reagents under specified condition. Do not use after expiration date.

2. Do not use the container and auxiliaries included in this kit for other purposes.

3. Do not mix reagents of different lot for use.

4. Do not add to the reagent being used even if it is the same lot number.

Disposal

1. All specimens, as well as all instruments (e.g. test tubes) that come in contact with the specimens, must be treated by the following methods, or they must be treated according to the manual for infectious medical waste provided in each facility.

・Sterilize with an autoclave, subjecting them to high pressure saturated steam at 121 °C for more than 20 minutes. Do not process waste containing sodium hypochlorite solution with an autoclave.

・Immerse at least one hour in sodium hypochlorite solution (active chloride concentration of over 1000 ppm).

2. This reagent contains sodium azide. Sodium azide can react with lead pipe and/or steel pipe and can generate explosive metal azide. Make sure to use plenty of water at disposal. Concentration of sodium azide in R-2 is 0.05%.

13. INFORMATION FOR AUTOANALYZERS

❖ **For Hitachi Model**

Calculation Method		Two point
Temperature		37°C
Volume (µL)	Specimen	2.4
	R1	120
	R2	120
Wavelength (nm)	Main	570
	Sub	800
Measurement (cycle)	Point 1	10
	Point 2	19
	Point 3	34
Calibration type		Spline
Unit		mg/dL

14. OTHER INSTRUCTIONS AND CAUTION

- Results may differ depending on the sample/reagent ratio. Adjust parameters for different analyzer.

- Prepare the calibration curve on the day of determination.

15. PACKING AND KIT CONFIGURATION

Code	Package	Test/Kit*	Test/Kit**
11C014A	1x60mL; 1x60mL	356	540
11C014A2	2x60mL; 2x60mL	712	1080
11C014A3	3x60mL; 3x60mL	1068	1620
11C024A	4x60mL; 4x60mL	1424	2160
11C014A5	5x60mL; 5x60mL	1780	2700
11C014A6	6x60mL; 6x60mL	2136	3240
11C014	1x90mL; 1x90mL	530	810
11C014-2	2x90mL; 2x90mL	1060	1620
11C024	3x90mL; 3x90mL	1590	2430
11C014-4	4x90mL; 4x90mL	2120	3240
11C014-5	5x90mL; 5x90mL	2650	4050

* For middle-scale automatic analyzers such as: SK300; BS series; BA200; BA400. Chemwell Series; Dirui Series; Biolyzer series, HumanStar 300, Erba Series; Bioelab Series, BX 3010; Pictus P500;…

** For large-scale automatic analyzers such as: CA800; CA400; Randox Imola; Randox Modena+; BM 6010; Biolis50i; SK500; AU Series; Pictus P700; C series; Ci series; HumanStar 600; Kenolab series ...

The above-mentioned test's number are calculated base on technical specifications of each analyzer. The real number of test per kit may higher than the calculation's number.

The above-mentioned test's number cover the loss of the dead volume of reagent bottles but not cover the loss of Calibrator and Control.

Please feel free to contact authorized distributor for further confirmation.

16. REFERENCES

1. P.J.H. Jones, T.C. Rideout,in Comprehensive Biotechnology (Third Edition), 2011
2. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices, EP05-A2, 2004
3. CLSI EP17 · Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition, 2017
4. In house data, UMA Diagnostics

17. MANUFACTURER

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