BECKMAN COULTER

IMMAGE Immunochemistry Systems

Instructions For Use

CCRP
High Sensitivity Cardiac C-Reactive Protein

REF A38656

For *In Vitro* Diagnostic Use

FOR PROFESSIONAL USE ONLY

This document is for use with IMMAGE 800 Immunochemistry System only

Rx Only

PRINCIPLE

INTENDED USE

High Sensitivity Cardiac C-Reactive Protein (CCRP) reagent, when used in conjunction with IMMAGE 800 Immunochemistry Systems and Calibrator 5 Plus, is intended for the quantitative determination of C-reactive protein in human serum or plasma by rate turbidimetry.

CLINICAL SIGNIFICANCE

Measurement of C-reactive protein (CRP) aids in evaluation of stress, trauma, infection, inflammation, surgery, and associated diseases.[1,2,3,4,5,6] Cardiac CRP assays are indicated for use as an aid in the identification and stratification of individuals at risk for future cardiovascular disease.[7] When used in conjunction with traditional clinical laboratory evaluation of acute coronary syndromes, CRP may be useful as an independent marker of prognosis for recurrent events in patients with stable coronary disease or acute coronary syndrome.

SUMMARY AND EXPLANATION

Blood levels of C-reactive protein are known to rise rapidly from normal baseline levels of < 0.3 mg/dL to as high as 50 mg/dL as part of the body`s non-specific inflammatory response to infection or injury.[1,2,3,4,5,6] In more recent years, the utility of measuring CRP has expanded from its historical use as a sensitive marker of acute inflammation to include assessment of cardiac events and risk.

A prognostic value for measuring CRP has been determined from studies with cardiac patients where elevated levels of CRP were associated with a higher risk of having a future cardiac event.[8,9,10,11] Elevated levels of CRP have been associated with poor prognosis in cases of stable angina, unstable angina and myocardial infarction.[8,9,10,11]

Cardiac disease is believed to be the end result of interplay between minor changes in the cardiovascular endothelium and the corresponding inflammatory response to these changes.[12] The ability to measure CRP at extremely low concentrations has raised the possibility of using CRP to detect early inflammatory responses and potentially detect cardiac disease in the preclinical stages. Recent evidence supporting this potential application has shown that high baseline values of CRP in individuals without a history of cardiac disease were associated with an increased incidence of subsequent cardiac events.[13,14]

The Centers for Disease Control and the American Heart Association (CDC/AHA) recommends the following cardiovascular disease risk assessment guidelines for CRP.[15, 16]

Table 1.0 CARDIOVASCULAR RISK CLASSIFICATION

RISK LEVEL	CRP (mg/L)	CRP (mg/dL)
Low	< 1.0	< 0.10
Average	1.0 – 3.0	0.10 – 0.30
High	> 3.0	> 0.30

It is important to note that baseline CRP values are known to be influenced by various non-pathological factors (age, gender, obesity, hormone replacement therapy, smoking) and a single measurement may lead to an erroneous assessment of early cardiac inflammation.[17,18,19,20,21] Increases in CRP levels are non-specific and should not be interpreted without a complete clinical history. It is recommended, therefore, that any estimations of inflammation be based on changes in CRP values from multiple measurements and be used in conjunction with the values of other cardiac risk indicators (i.e., HDL, cholesterol, etc.).

METHODOLOGY

The IMMAGE 800 Immunochemistry Systems CCRP reagent is based on the highly sensitive Near Infrared Particle Immunoassay rate methodology. An anti-CRP antibody-coated particle binds to CRP in the patient sample resulting in the formation of insoluble aggregates causing turbidity. The rate of aggregate formation is directly proportional to the concentration of CRP in the sample.

CHEMICAL REACTION SCHEME

E011315L.EPS

SPECIMEN

TYPE OF SPECIMEN

Serum samples are recommended. Plasma samples (EDTA, Lithium Heparin, and Sodium Heparin) can be used.

Serum or plasma samples should be collected in the manner routinely used for any clinical laboratory test.[6] Freshly drawn serum or plasma from a fasting individual is preferred. Anticoagulants tested are listed in the PROCEDURAL NOTES section of this instructions for use.

SPECIMEN STORAGE AND STABILITY

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.[22]
2. If serum and plasma samples are not assayed within 8 hours, samples should be stored at +2°C to +8°C. If serum and plasma samples are not assayed within 48 hours, samples should be stored frozen at -15°C to -20°C for up to 36 months. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.[22,23]

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE VOLUME

For sample volumes refer to the Sampling Template.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this instructions for use.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

KIT COMPONENTS	QUANTITY
CCRP Cartridge	2
Antibody	
Evaporation Caps	4
CCRP Reagent Bar Code Card	1

INITIAL VOLUMES OF SAMPLE AND REAGENTS IN THE CUVETTE

Sample Volume	4.5 µL
Total Reagent Volume	209 µL
Antibody-coated particle	42 µL
Buffer 4	125 µL
Diluent	42 µL

REACTIVE INGREDIENTS

REAGENT CARTRIDGE CONSTITUENTS	VOLUME
CRP Antibody (particle bound goat and mouse anti-CRP antibody)	7.6 mL
Diluent	7.6 mL
Sodium Azide (used as a preservative)	< 0.1% (w/w)
Bovine Serum Albumin	0.1% (w/v)

Also non-reactive chemicals necessary for optimal system performance.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

⚠ CAUTION

Although not composed of substances of human origin, this product may come in contact with human serum during processing. This material and all patient samples should be handled as though capable of transmitting infectious disease. The United States Food and Drug Administration recommends such samples be handled as specified in the Centers for Disease Control`s Biosafety Level 2 guidelines.[24]

GHS HAZARD CLASSIFICATION

High Sensitivity Cardiac C-Reactive Protein Reagent (Compartment B)	WARNING	
		
		
	H316	Causes mild skin irritation.
	H319	Causes serious eye irritation.
	H411	Toxic to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
	P337+P313	If eye irritation persists: Get medical advice/attention.
		1-(3-Dimethylaminopropyl)-3-Ethylcarbodiimide Hydrochloride < 1%
		Tris(hydroxymethyl)– aminomethane 1 - 5%
		Choline Chloride 1 - 5%
		Sodium Trichloroacetate 1 - 10%
		Ethylenediaminetetraacetic Acid, Dipotassium Salt, Dihydrate 1 - 5%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

IMMAGE Immunochemistry Systems Wash Solution
IMMAGE Immunochemistry Systems Diluent 1
IMMAGE Immunochemistry Systems Buffer 4
Calibrator 5 Plus
Centrifuge capable of 90,000 x g
At least two levels of control material

REAGENT PREPARATION

1. Invert cartridge gently before removing screw caps.

2. Remove screw caps from reagent cartridges. Check each cartridge for bubbles and remove any bubbles present.
3. Place evaporation caps on both reagent cartridge compartments before loading the cartridge on the instrument.
4. Reagent cartridges should be stored upright and can be removed from the refrigerator and used immediately.
5. Mix all buffers and diluents thoroughly by inversion. Remove screw cap from container. Check each container for bubbles and remove any bubbles present. Place evaporation cap on container before loading the container on the instrument.

ACCEPTABLE REAGENT PERFORMANCE

Acceptability of a reagent is determined from the successful performance of quality control testing, as defined in the QUALITY CONTROL section of this instructions for use.

REAGENT STORAGE AND STABILITY

Storage conditions other than those recommended may cause erroneous results.

Reagent Cartridges

1. Return all reagent cartridges to the refrigerator (+2°C to +8°C) upon completion of the daily workload.
2. The CCRP reagents are stable for 30 days with the evaporation caps in place.
3. The CCRP reagent is stable until the expiration date on the label if stored at +2°C to +8°C with the screw caps in place.

Diluent 1 and Buffer 4

1. Diluent 1 and Buffer 4 are stable on the system for 30 days with the evaporation cap in place.
2. Diluent 1 and Buffer 4 are stable until the expiration date on the label if stored at room temperature with the screw cap in place.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

Calibrator 5 Plus

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

The calibrator is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C.

⚠ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.[24]

Calibrator storage location:

CALIBRATION INFORMATION

1. The IMMAGE 800 Immunochemistry Systems calibration is reagent lot specific.
2. The CCRP reagent lot should be recalibrated when changing Buffer 4 lot or following specific part replacements or maintenance procedures as defined in the IMMAGE 800 Immunochemistry Systems *Operations Manual*.
3. The IMMAGE 800 Immunochemistry System is designed for minimum calibration. Calibrations retained in system memory should be monitored by the performance of quality control procedures on each day of testing.
4. Calibration for CCRP is stable for 30 days.
5. The system will automatically perform a verification check during calibration and produce a calibration report. The system will alert the operator of a failured calibration. An explanation of any accompanying error message can be found in the TROUBLESHOOTING Section of the IMMAGE 800 Immunochemistry Systems *Operations Manual*.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

It is recommended that at least two levels of control material, normal and abnormal, be analyzed daily. Controls should also be run with each new calibration, with a new lot of reagent or buffer, and after specific maintenance or troubleshooting as detailed in the IMMAGE Immunochemistry Systems *Operations Manual*. More frequent use of controls or the use of additional controls is left to the discretion of the user based on work load and work flow.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

Table 2.0 Quality Control Material

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

1. After setup, load reagents onto the system as directed in the IMMAGE 800 Immunochemistry Systems *Operations Manual*.
2. Select chemistries to be calibrated, if necessary. Load bar coded calibrators, controls, and samples or program and load non-bar coded controls and samples for analysis as directed in the IMMAGE 800 Immunochemistry Systems *Operations Manual*.
3. Follow the protocols for system operation as directed in the IMMAGE 800 Immunochemistry Systems *Operations Manual*.

CALCULATIONS

The IMMAGE 800 Immunochemistry System will automatically calculate results.

REPORTING RESULTS

REFERENCE INTERVALS

The reference interval values were established using the IMMAGE Immunochemistry Systems CCRP test, for a population of 615 apparently healthy, non-smoking, ≥ 18 years of age, male and female adults from a Southern California blood bank.

Each laboratory should establish its own reference interval(s) based on its patient population.

Table 3.0 Reference intervals

INTERVALS	CONVENTIONAL UNITS	S.I. UNITS
Beckman Coulter	< 0.744 mg/dL (in 95% of the population tested)	< 7.44 mg/L (in 95% of the population tested)

INTERVALS	CONVENTIONAL UNITS	S.I. UNITS
Laboratory		

Refer to References (25,26) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

UNITS AND CONVERSION FACTOR

Results for the CCRP test are reported in default units of mg/L. Metric conversion within the same unit category will occur automatically if a new unit is selected. A conversion factor must be entered when selecting a unit category different from the default. For example, 0.2 mg/L will be converted to 0.02 mg/dL (mg/L ÷ 10 = mg/dL).

Refer to the System Setup section of the IMMAGE 800 Immunochemistry Systems *Operations Manual* for more detailed information on units and conversion factors.

PROCEDURAL NOTES

ANTICOAGULANT TEST RESULTS

The following anticoagulants were assessed by Deming regression analysis with a minimum of 50 paired human serum and plasma samples. Values of serum (X) ranging from 0.34 mg/L to 58.5 mg/L were compared with the values for plasma (Y) yielding the following results.

Table 4.0 Anticoagulant Test Results

ANTICOAGULANT	LEVEL OF ANTICOAGULANT TESTED	DEMING REGRESSION ANALYSIS (mg/L)
Lithium Heparin	14 Units/mL	Y = 1.050X - 0.2; r = 0.993
Sodium Heparin	14 Units/mL	Y = 1.028X - 0.3; r = 0.997
EDTA	1.5 mg/mL	Y = 1.042X - 0.2; r = 0.993

LIMITATIONS

Increases in CRP levels are non-specific and should not be interpreted without a complete clinical history. It is recommended that a baseline be determined on individual patients for comparison. Estimations of inflammation should be based on the changes in CRP values from multiple measurements and used in conjunction with the values of other cardiac risk indicators (i.e., HDL, cholesterol, etc.).

AHA/CDC Expert Panel Recommendations[16]: Screening the entire adult population is not recommended. CRP is not a substitute for traditional cardiovascular risk factors. Acute coronary syndrome management should not depend on CRP measurements. When being used for risk assessment, patients with persistently unexplained CRP levels above 10 mg/L should be evaluated for other non-cardiovascular origins. Testing for any risk assessment should not be performed while there is indication of infection, systemic inflammation, or trauma. Secondary prevention measures should be based on global risk assessment and not depend on CRP. Serial testing of CRP should not be used to monitor effects of treatment. The average of CRP results repeated optimally two weeks apart should be used in performing risk assessment on metabolically stable patients.

INTERFERENCES

1. The following substances were tested in serum for interference with this methodology at the initial dilution:

Table 5.0 Interferences

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT
Bilirubin	Porcine	5 – 40 mg/dL	None
Lipid	Human Triglyceride	126 – 1,000 mg/dL	None[a]
Hemoglobin	Human	100 – 650 mg/dL	None

a Quantitation of CCRP by turbidimetry may not be possible in lipemic specimens or may produce inaccurate results, due to the extreme light scattering properties of the sample. Lipemic specimens should be delipidated by ultra centrifugation (90,000 x g for 10 minutes) prior to determination of CCRP concentration.

2. Dust particles or other particulate matter (i.e. debris and bacteria) in the reaction solution may result in extraneous light-scattering signals, resulting in variable sample analysis.
3. For assays employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Human anti-mouse antibodies may be present in samples from patients who have received immunotherapy or diagnostic procedures utilizing monoclonal antibodies or in individuals who have been regularly exposed to animals.[27,28] Additionally, other heterophile antibodies, such as human anti-goat antibodies may be present in patient samples. Interpretation of results should be done in the context of the overall clinical presentation of the patient, including symptoms, clinical history, data from additional tests and other appropriate information.

PERFORMANCE CHARACTERISTICS

NOTE ON PERFORMANCE CHARACTERISTICS

Performance characteristics are based on the initial analytical range. The instrument automatically dilutes samples to be within the initial analytical range. Refer to IMMAGE Immunochemistry Systems Operations Manual for more information.

ANALYTIC RANGE

The CCRP test is designed to detect concentrations of this analyte using an initial undiluted (neat) sample.

Table 6.0 Analytical Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Serum or Plasma	Initial: 0.02 – 6.0 mg/dL Extended: 0.02 – 144 mg/dL	Initial: 0.2 – 60.0 mg/L Extended: 0.2 – 1,440 mg/L

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 7.0 Reportable Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

ANALYTICAL SENSITIVITY

Analytical sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Analytical sensitivity for CCRP determination is 0.06 mg/L (0.006 mg/dL).

FUNCTIONAL SENSITIVITY

Functional sensitivity is defined as the lowest concentration that can be measured with an interassay CV of 20%. Functional sensitivity is estimated to be = 0.11 mg/L (= 0.011 mg/dL).

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of samples to an accepted clinical method. Values obtained for CCRP using the IMMAGE 800 Immunochemistry Systems CCRP test were compared to the values obtained using a commercially available automated nephelometric assay (NIA) method. Both normal and abnormal samples were included in the analysis.

Table 8.0 Equivalency Values

	Initial Analytical Range (0.23 – 66.2 mg/L)	Cardiac Range (0.23 – 10.1 mg/L)
N	157	98
Slope	0.965	1.013
Intercept	0.334	- 0.026
Mean (IMMAGE)	12.703	2.717
Mean (NIA)	12.815	2.708
Correlation Coefficient (r)	0.996	0.994

Refer to References [29,30] at the end of this instructions for use for guidelines on performing equivalency testing.

PRECISION

A properly operating IMMAGE 800 Immunochemistry System should exhibit imprecision values less than or equal to the maximum performance limits listed below. Maximum performance limits were derived by an examination of the precision of various methods, proficiency test summaries, and literature sources.

Table 9.0 Maximum Performance Limits

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE[a]		% CV
		(mg/dL)	(mg/L)	(mg/dL)	(mg/L)	
Within-run	Serum/Plasma	0.005	0.05	0.1	1.0	5.0
Total	Serum/Plasma	0.0075	0.075	0.1	1.0	7.5

a When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Comparative performance data for the IMMAGE 800 Immunochemistry System evaluated using the CLSI/NCCLS Approved Guideline EP5-A2 appears in the table below.[31] Each laboratory should characterize their own instrument performance for comparison purposes.

Table 10.0 Typical Imprecision Values

TYPE OF PRECISION	SAMPLE	Data Points[a]	Test Mean Value (mg/L)	SD (mg/L)	% CV
Within-run	Serum Level 1	80	0.807	0.0229	2.8
	Serum Level 2	80	13.56	0.4109	3.0
	Serum Level 3	80	51.538	1.7181	3.3
Total	Serum Level 1	80	0.807	0.0279	3.5
	Serum Level 2	80	13.56	0.4248	3.1
	Serum Level 3	80	51.538	2.1933	4.3

a The serum point estimate is based on the data from 1 system, run for 20 days, 2 runs per day, 2 observations per run on an instrument operated and maintained according to the manufacturer`s instructions.

Refer to References (29, 31) for guidelines on performing precision testing.

NOTICE

These degrees of precision were obtained in typical testing procedures and are not intended to represent performance specifications for this test procedure.

ADDITIONAL INFORMATION

For more information, refer to the IMMAGE 800 Immunochemistry Systems *Operations Manual*.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REVISION HISTORY

Revision AB

Added new language requirement: Norwegian.

Revision AC

Updated corporate address.

Revision AD

Added Revision History

Revision AE

Added new language requirement: Czech, and Korean.

Revision AF

Revised Reagent Cartridge Constituents Volume.

Revision AG

Added GHS Classification information

Revision AH

Updates to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AJ

Added new language requirement: Brazilian Portuguese.

Revision AK

Added new language requirement: Dutch, Romanian, and Slovak. Additional changes to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AL

Removed references to "Chemistry Reference Manual", CE mark and EC Rep name and address.

Revision of sample stability information, minor instructional clarification and corrections throughout, and updates to comply with requirements per Beckman Coulter Global Labeling Policy.

SYMBOLS KEY

Table 11.0

REF	Catalogue Number	IVD	In Vitro Diagnostic
CONTENTS	Contents		Temperature limit
	Manufacturer		Expiration Date
LOT	Batch code	SDS	Safety Data Sheet
CE	CE Mark		Consult Instructions for Use
EC REP	Authorized Representative in the European Community		Date of Manufacture
	Caution		Biological risks
REAGENT CART	Reagent Cartridges	CAPS	Caps
BAR CODE CARD	Bar Code Card	WARNING	WARNING
SN	Serial Number		
Made in USA of US and Foreign Components		Made in USA of US and Foreign Components	

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