BIO TECHNOLOGY boditech

Cardiac

ichroma™ hsCRP

INTENDED USE

ichroma™ hsCRP is a fluorescence immunoassay (FIA) for the quantitative determination of CRP (C-Reactive Protein) in human whole blood /serum/plasma. It is useful as an aid in management and monitoring of risk of cardiovascular diseases.

For *in vitro* diagnostic use only.

INTRODUCTION

The C-Reactive Protein (CRP) is synthesized by the liver in response to interleukin-6 and well known as one of the classical acute-phase reactants and as a marker of inflammation. It has recently been suggested that a marker of inflammation, along with serum cholesterol, may be critical component in the development and progression of atherosclerosis[1,2]. A growing body of evidence has supported the idea that cardiovascular diseases including coronary heart disease, ischemic stroke, and acute myocardial infarction, develop, at least in part, because of a chronic low-level CRP of the vascular endothelium[3,4]. Apparently, high-sensitivity CRP (hsCRP) is emerging as the strongest and most independent predictive risk factor for atherosclerosis and CVD[5,6]. American Heart Association (AHA) and the Centers for Disease Control and Prevention (CDC) issued a statement regarding use of C-reactive protein to assess risk of cardiovascular diseases.

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized antibodies on a test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for ichroma™ tests to show CRP concentration in the sample.

COMPONENTS

ichroma™ hsCRP consists of 'cartridges' and 'detection buffer tubes'.

- The cartridge contains the membrane called a test strip which has anti-CRP at the test line, CRP antigen at the antigen line and rabbit IgG at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a cartridge box.
- The detection buffer contains anti-CRP-fluorescence conjugate, anti-rabbit IgG-fluorescence conjugate, and sodium azide as a preservative in phosphate buffered saline (PBS). It is pre-dispensed in tubes. The detection buffer tubes are packaged in a detection buffer box and further packed in a styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detection buffer and ID chip) must match with each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges or detection buffer tubes. A cartridge should be used for testing one sample only. A detection buffer tube should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge, detection buffer tube and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detection buffer tubes and sample collectors should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detection buffer tube contains sodium azide (NaN_3), and it may cause certain health issues like convulsions, low blood pressure, low heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in **ichroma™ hsCRP** when biotin concentration in the sample was below upto 3,500 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **ichroma™ hsCRP** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ hsCRP** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2 EDTA, K_3 EDTA, Sodium heparin, Lithium heparin, Sodium citrate

- **The sample collector should be used when the following conditions are met.**
  - The sample collector provided with the kit is recommended to obtain correct test result.
  - Whole blood should be immediately tested after collection.
  - Excess whole blood around the sample collector should be wiped off.
  - In order to avoid cross-contamination, please do not re-use the sample collector for multiple samples.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-

reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigens to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigens with time and/or temperature may also cause false negative result as it makes the antigens unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage temperature	Shelf life	Note
Cartridge	2 - 30°C	20 months	Disposable
Detection buffer tube	2 - 8°C	20 months	Disposable

- After the cartridge pouch is opened, the test should be performed immediately.

MATERIALS SUPPLIED

REF CFPC-6

Components of **ichroma™ hsCRP**
- Cartridge box:
  - Cartridge 25
  - Sample collector 25
  - ID chip 1
  - Instructions for use 1
- Detection buffer box:
  - Detection buffer tube 25

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately with **ichroma™ hsCRP**.

Please contact our sales division for more information.
- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M3** REF FPRR035
  - **ichroma™-50 PLUS** REF FPRR036
- **Printer** REF FPRR007
- **Boditech hsCRP Control** REF CFPO-374

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ hsCRP** is human whole blood/serum/plasma.
- It is recommended to test the sample within 24 hours after collection when collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection. of whole blood.
- The samples (whole blood, serum, plasma) may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples (serum, plasma) should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.
- Whole blood sample may be used to collect according to below:

  ① Wear disposable gloves and protective equipment for safety.

  ② Open the zipper bag which has sample collectors.

  ③ Take out the sample collector and check for damage or contamination.

  ④ Touch the surface of blood with the sample collector.

  ⑤ Fill it with blood completely.

  (Make sure that no air bubbles are present in the sample collector. Do not get blood on the surface of the sample collector. If the blood gets on the surface of the sample collector, remove it gently with gauze.)

TEST SETUP

- Check the contents of **ichroma™ hsCRP**: Sealed cartridges, detection buffer tubes, sample collectors, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the detection buffer tube as well as the ID chip.
- If the sealed cartridge and the detection buffer have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for ichroma™ tests.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the instrument for ichroma™ tests operation manual for complete information and operating instructions.**

TEST PROCEDURE

▶ **ichroma™ Reader, ichroma™ II, ichroma™ M3**

Multi test mode

1) Make a puncture on the top of the detection buffer tube by inserting an empty sample collector.
2) Take 10 µL of sample (whole blood/serum/plasma/control) using a sample collector.
3) Assemble the sample collector and the detection buffer tube into one.
4) Shake 10 times or more until the sample out of the sample collector by inversion. (The sample mixture must be used immediately. Do not exceed 30 seconds.)
5) Remove the cap off the top of assembled tube. Discard two drops of reagent onto the paper towel before applying to the cartridge.

6) Apply only two drops of the mixture into the sample well of the cartridge.
7) Leave the cartridge at room temperature for 3 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
8) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
9) Press the 'Select' button or tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
   (ichroma™ M3 will start the test automatically after inserting.)
10) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
11) Read the test result on the display screen of the instrument for ichroma™ tests.

Single test mode

1) The test procedure is same with the 'Multi test mode 1) – 6)'.
2) Insert the sample-loaded cartridge into the holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
3) Press the 'Select' or tap the 'Start' button on the instrument for ichroma™ tests.
   (ichroma™ M3 will start the test automatically after inserting.)
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 3 minutes.
5) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ ichroma™ III

1) The test procedure is same with the 'Single test mode'.

▶ ichroma™-50 PLUS

1) Insert the tip array in the tip station.
2) Insert the detection buffer tubes in the reagent station and cover the reagent station to hold the detection buffer tubes in place.
3) Insert the cartridge magazine with the cartridges into the magazine station.
4) Insert the sample tube into the blood collection tube rack and load the blood collection tube rack into the sampling station (loading part).
5) Tap the button located in the upper side of the No. of test cartridge region to select the ID chip that you want to use.
6) When the selected cartridge slot is activated, set the number of the detection buffer tube by tapping.
7) Set the number of pipette tips by tapping.
8) Tap the 'Start' button on the left upper of the main screen to start test.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays CRP concentration of the test sample in terms of mg/L.
- Working range: 0.1 - 10 mg/L
- Reference value

Range of CRP [mg/L]	Definition
< 1	Low risk in cardiovascular disease
1 ~ 3	Average risk in cardiovascular disease
> 3	High risk in cardiovascular disease

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **ichroma™ hsCRP**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical Sensitivity**
  - Limit of Blank (LoB) 0.012 mg/L
  - Limit of Detection (LoD) 0.029 mg/L
  - Limit of Quantitation (LoQ) 0.10 mg/L

- **Analytical Specificity**
  - **Interference**
    Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ hsCRP** test results did not show any significant interference with these materials.

Interferents	Concentration
Bilirubin (Unconjugated)	257 µmol/L
Albumin	60 g/L
D-Glucose	1,000 mg/dL
Hemoglobin	10 g/L
L-Ascorbic acid	5.25 mg/dL
Triglyceride mixture	1,500 mg/dL
K_2 EDTA	3.4 µmol/L
K_3 EDTA	3.4 µmol/L
Sodium heparin	3000 U/L
Lithium heparin	3000 U/L
Sodium citrate	2 mg/mL
Biotin	3,500 ng/L

  - **Cross reactivity**
    Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ hsCRP** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Concentration
Troponin complex	1,000 ng/mL
D-Dimer	20,000 ng/mL
NT-proBNP	1,000 ng/mL

Myoglobin	500 mg/L
CK-MB	200 mg/L

- **Precision**

- **Single-site study**

Repeatability (within-run precision)
within-laboratory precision (Total precision)
Lot to lot precision

3 Lots of **ichroma™ hsCRP** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Conc. [mg/L]	Repeatability			Within-laboratory precision		
	Mean [mg/L]	SD	CV (%)	Mean [mg/L]	SD	CV (%)
0.5	0.5	0.03	6.7	0.5	0.03	6.5
1.5	1.5	0.08	5.4	1.51	0.09	5.7
5	4.98	0.29	5.9	5.00	0.30	6.0

Conc. [mg/L]	Lot to Lot precision		
	Mean [mg/L]	SD	CV (%)
0.5	0.50	0.03	6.2
1.5	1.51	0.09	6.2
5	5.02	0.31	6.1

- **Multi-site study**

Reproducibility

1 Lot of **ichroma™ hsCRP** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Conc. [mg/L]	Reproducibility		
	Mean [mg/L]	SD	CV (%)
0.5	0.49	0.03	6.8
1.5	1.49	0.09	6.2
5	5.01	0.32	6.3

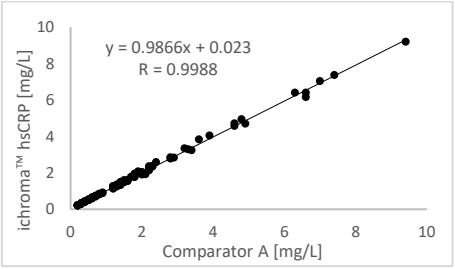
- **Accuracy**

The accuracy was confirmed by testing with 3 different of **ichroma™ hsCRP**. The tests were repeated 10 times at each concentration of the control standard.

Conc. [mg/L]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
10.00	9.88	10.08	10.32	10.09	100.9
8.02	7.74	8.03	8.03	7.94	99.0
6.04	5.97	5.85	6.02	5.95	98.5
4.06	4.04	4.01	4.06	4.04	99.4
2.08	2.03	2.09	2.14	2.09	100.4
0.10	0.10	0.10	0.10	0.10	102.1

- **Comparability**

CRP concentration of 103 clinical samples were quantified independently with **ichroma™ hsCRP (ichroma™ II)** and **comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.



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Note: Please refer to the table below to identify various symbols

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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