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Cardiac

AFIAS hsCRP

INTENDED USE

AFIAS hsCRP is a fluorescence immunoassay (FIA) for the quantitative determination of CRP 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 AFIAS tests to show CRP concentration in the sample.

COMPONENTS

AFIAS hsCRP consists of a 'cartridges'.

- Each sealed aluminum pouch contains two cartridges.
- Each cartridge packaged in an aluminum pouch has three components including a cartridge part, a detector part and a detector diluent part.
- The cartridge part contains the membrane called a test strip, which has mouse monoclonal anti-CRP at the test line, CRP Ag at the antigen line and Rabbit IgG at the control line.
- The detector part contains a granule containing mouse monoclonal anti-human CRP fluorescence conjugate, anti-rabbit IgG fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline (PBS).
- The diluent part contains tween 20 and sodium azide as a preservative in phosphate buffered saline (PBS).

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instructions for use'.
- Use fresh samples only and avoid direct sunlight.
- Lot numbers of all the test components (cartridge and ID chip) must match each other.
- Do not interchange the test components between different lots or use test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges and pipette tips. A cartridge should be used for testing one sample only.
- The cartridge should remain sealed in its aluminum pouch until just before use. Do not use the cartridge, if the 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 and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for AFIAS tests may generate slight vibration during use.
- Used cartridges and pipette tips should be handled carefully and disposed by an appropriate method in accordance with relevant local regulations.
- The cartridge contains sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure and 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 **AFIAS hsCRP** when biotin concentration in the sample was below 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.
- **AFIAS hsCRP** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS hsCRP** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

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

- **C-tip should be used when the following conditions are met.**
  - C-tip provided with the kit is recommended to obtain correct test result.
  - Whole blood should be immediately tested after collection.
  - Do not perform a test with C-tip on General mode. It might cause an erroneous result.
  - Excess whole blood around the C-tip should be wiped off.
  - In order to avoid cross-contamination, please do not re-use C-tip for multiple samples.
  - AFIAS cartridge should be inserted and positioned in the cartridge holder prior to the blood sample collection.
  - While collecting blood, be careful not to create air bubbles in the C-tip.

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 antigen 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 antigen with time and/or temperature may also cause false negative result as it makes the antigen 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

Component	Storage condition: Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Unopened
		1 month	Resealed

- Return an unused cartridge to the spare cartridge zipper bag containing the desiccant pack. Reseal along entire edge of zip-seal.

MATERIALS SUPPLIED

REF SMFP-78

Components of **AFIAS hsCRP**

- Cartridge box:
  - Cartridge 24
  - Pipette tip (zipper bag) 24
  - C-tip (10 µL) 24
  - Spare cartridge zipper bag 1
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **AFIAS hsCRP.**

Please contact our sales division for more information.

- Instrument for AFIAS tests
  - **AFIAS-1** REF FPRR019
  - **AFIAS-3** REF FPRR040
  - **AFIAS-6** REF FPRR020
  - **AFIAS-10** REF FPRR038
- **Boditech hsCRP Control** REF CFPO-374
- **Boditech hsCRP Calibrator** REF CFPO-381

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS hsCRP** is human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- 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 24 hours at 2-8 °C prior to being tested. If testing will be delayed more than 24 hours, samples should be frozen at - 20 °C
- The samples (serum, plasma) stored frozen at - 20 °C for 3 months 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.
- Collection of whole blood sample using C-tip
  ① Clean the area with a pre-injection swab.
  ② Pierce with a sterile lancet.
  ③ Wipe away the first drop of blood.
  ④ Gently massage the around the pricked fingertip for the second drop.
  ⑤ Hold a C-tip horizontally and touch the blood drop with the tip of the C-tip.
  ⑥ Capillary action will automatically draw the blood sample to C-tip and stop.
  ⑦ Wipe off any excess blood around the tip.
  ⑧ Double-check if whole blood is filled accurately in the C-tip and AFIAS reader is ready for a test on the 'C-tip mode'.

TEST SETUP

- Check the components of the **AFIAS hsCRP** as described below. : Cartridges, pipette tips, an ID chip, a spare cartridge zipper bag and an instructions for use.
- If the sealed cartridge has 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 AFIAS tests.
- Empty the tip box.
- Insert the ID chip into the 'ID chip port'.

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

TEST PROCEDURE

▶ **AFIAS-1, AFIAS-3, AFIAS-6**

General mode

1) Insert a cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.
3) Select the 'General mode' in the instrument for AFIAS tests.
4) Take 100 µL of the sample (whole blood/serum/plasma) using a pipette and dispense it into the sample well of the cartridge.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 3 minutes.

C-tip mode

1) Insert the cartridge into the cartridge holder.
2) Take 10 µL of whole blood with a C-tip.
3) Insert the whole blood-filled C-tip into the tip hole of the cartridge.

4) Select the 'C-tip mode' in the instrument for AFIAS tests.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 3 minutes.

▶ **AFIAS-10**

Normal mode

1) Insert a cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.
3) Tap the 'Load' button of the bay that holds the cartridge with the tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
4) Insert the sample tube into the tube rack.
5) Insert the tube rack into the loading part of the sampling station.
6) Tap the 'Start' button on the screen.

Emergency mode – General tip

1) The test procedure is same with the 'Normal mode 1) – 3)'.
2) Convert the 'Emergency mode' in AFIAS-10.
3) Select the tip type (general tip) on the screen.
4) Select the sample type (whole blood/serum/plasma) on the screen.
5) Take 100 µL of sample using a pipette and dispense it into the sample well of the cartridge.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 3 minutes.

Emergency mode – C-tip

1) Insert a cartridge into the cartridge holder.
2) Take 10 µL of whole blood using a C-tip.
3) Insert the C-tip with sample into the tip hole of the cartridge.
4) Tap the 'Load' button of the bay that holds the cartridge with a tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
5) Convert the 'Emergency mode' in AFIAS-10.
6) Select the tip type (C-tip) on the screen.
7) Tap the 'Start' button on the screen.
8) The test result will be displayed on the screen after 3 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays hsCRP concentration of the test sample in terms of 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

- Working range : 0.1-10 mg/L

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 **AFIAS 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**
  - **Cross-reactivity**

Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **AFIAS hsCRP** test results did not show any significant cross-reactivity with these biomolecules.

Cross reactivity substance	Concentration
Troponin complex	1,000 ng/mL
D-dimer	20,000 ng/mL
NT-proBNP	1,000 ng/mL
Myoglobin	200 ng/mL
CK-MB	100 ng/mL

  - **Interference**

Interferents listed in the following table were added to the test sample–at the concentration mentioned below. **AFIAS hsCRP** test results did not show any significant interference with these materials.

Interferent	Concentration
Bilirubin (Unconjugated)	257 µmol/L
Cholesterol	6.47 mmol/L
D-Glucose	1,000 mg/dL
Hemoglobin	2 g/L
L-Ascorbic acid	170 µmol/L
Triglyceride mixture	500 mg/dL
K_2EDTA	3.4 µmol/L
K_3EDTA	3.4 µmol/L
Sodium heparin	3,000 U/L
Lithium heparin	3,000 U/L
Sodium citrate	2 mg/mL
Biotin	3,500 mg/mL

- **Precision**
  - Single-site study

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

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

hsCRP [mg/L]	Repeatability				Within-laboratory precision			
	N	Mean [mg/L]	SD	CV (%)	N	Mean [mg/L]	SD	CV (%)
0.5	40	0.49	0.03	6.55	80	0.50	0.03	6.55
1.5	40	1.51	0.10	6.51	80	1.51	0.10	6.38
5	40	5.06	0.30	6.00	80	5.04	0.31	6.17

hsCRP [mg/L]	Lot to Lot precision			
	N	Mean [mg/L]	SD	CV (%)
0.5	240	0.50	0.03	6.43
1.5	240	1.50	0.09	6.17
5	240	5.03	0.32	6.39

  - Multi-site study

Reproducibility

1 Lot of **AFIAS 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.

hsCRP [mg/L]	Reproducibility			
	N	Mean [mg/L]	SD	CV (%)
0.5	75	0.50	0.03	5.92
1.5	75	1.48	0.09	3.35
5	75	4.97	0.30	6.02

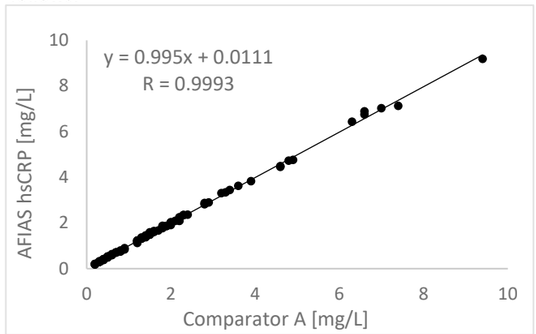
- **Accuracy**

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

hsCRP [mg/L]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
10.00	10.09	10.03	9.77	9.96	99.6
8.02	8.07	8.17	8.02	8.09	100.9
6.04	6.04	5.98	6.04	6.02	99.7
4.06	4.00	4.15	4.09	4.08	100.5
2.08	2.13	2.02	2.16	2.10	101.0
0.10	0.10	0.10	0.10	0.10	100.5

- **Comparability**

CRP concentration of 103 clinical samples were quantified independently with **AFIAS hsCRP (AFIAS-6)** 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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