Cardiac

AFIAS CK-MB

INTENDED USE

AFIAS CK-MB is a fluorescence immunoassay (FIA) for the quantitative determination of CK-MB (Creatine Kinase Isoenzyme-MB) in human whole blood/serum/plasma. It is useful as an aid in management and monitoring of acute myocardial infarction (AMI) and acute coronary syndrome (ACS).

For *in vitro* diagnostic use only.

INTRODUCTION

Creatine Kinase (CK), also known as Creatine Phosphokinase or Phospho-creatine Kinase is an enzyme expressed by various tissues and cell types. Disruption of cell membranes due to hypoxia or other injury releases CK from the cellular cytosol into the systemic circulation. CK is a dimeric enzyme consisting of two subunits, which can be either B- (brain type) or M- (muscle type). These subunits associate to form three isoenzymic forms: CK-BB, CK-MM and CK-MB. These isoenzymes are expressed at different levels in various human tissues. Though CK-MM is the most abundant CK isoenzyme in the cardiac muscles, CK-MB constitutes about 20 % of the total CK in the cardiac muscle tissue. Elevated levels of total CK is not specific to the myocardial tissue and may be observed in patients with skeletal muscle injury and certain other disorders but as CK-MB is more specific to myocardial tissue, CK–MB levels along with total CK can be considered as an important diagnostic indicator of myocardial infarction. The concentration of CK-MB in the healthy adult is below 7.0 ng/ml but it shows great increases in several malignant diseases, mostly primary coronary syndrome, myocardial injury and infarction. CK-MB has been found to be more sensitive and earlier indicator of myocardial injury because it has a lower basal level and a much narrower normal range. Medical literature commonly reveals that following an acute myocardial infarction, CK-MB levels become elevated in 4 to 9 hours after the onset of chest pain, attain peak at 10 to 24 hours, and return to normal within 2 to 3 days. Use of CK-MB level as a percentage of total CK in the diagnosis of myocardial infraction is the most important clinical application of CK measurements in clinical chemistry.

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 CK-MB concentration in the sample.

COMPONENTS

AFIAS CK-MB consists of '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 diluent part.
- The cartridge part contains the membrane called a test strip which has anti-CK-MB at the test line, and streptavidin at the control line.
- The detector part contains anti-CK-MB-fluorescence conjugate, biotin-BSA-fluorescence conjugate, 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 only fresh samples 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 the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges. A cartridge should be used for testing one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use a 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 discarded by an appropriate method in accordance with relevant local regulations.
- The cartridge contains sodium azide (NaN_3), and it may cause certain health issues like convulsions, low blood pressure and 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 **AFIAS CK-MB** when biotin concentration in the sample was below 50 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 CK-MB** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS CK-MB** 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

LIMITATIONS 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

Component	Storage condition: Storage Temperature	Shelf life	Note
Cartridge	2 - 8 °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-13
Components of **AFIAS CK-MB**

- Cartridge box
  - Cartridge 24
  - Pipette tip (zipper bag) 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 with **AFIAS CK-MB**.
Please contact our sales division for more information.

- **Instrument for AFIAS tests**
  - **AFIAS-1** REF FPRR019
  - **AFIAS-3** REF FPRR028
  - **AFIAS-6** REF FPRR020
  - **AFIAS-10** REF FPRR038
- **Boditech Cardiac Control** REF CFPO-98
- **Boditech CK-MB Control** REF CFPO-243
- **Boditech Cardiac Calibrator** REF CFPO-110
- **Boditech CK-MB Calibrator** REF CFPO-269

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS CK-MB** 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 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.

TEST SETUP

- Check the components of the **AFIAS CK-MB** 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 sample (whole blood/serum/plasma /control) 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 12 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.
7) The test result will be displayed on the screen after 12 minutes.

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 the 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 12 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays CK-MB concentration of the test sample in terms of ng/mL.
- Reference value: 7 ng/mL
- Working range: 3-100 ng/mL

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 CK-MB**. 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)	0.69 ng/mL
Limit of Detection (LoD)	1.17 ng/mL
Limit of Quantitation (LoQ)	3.00 ng/mL

- **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 CK-MB** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Concentration
Troponin complex	1,000 ng/mL
NT-proBNP	1,000 ng/mL
Myoglobin	200 ng/mL
D-Dimer	20,000 ng/mL

- Interference
  Interferents listed in the following table were added to the test sample at the concentration mentioned below. **AFIAS CK-MB** 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
EDTA	3.4 µmol/L
Heparin	3000 U/L
Sodium citrate	2 mg/mL

- **Precision**

- Single-site study
  Repeatability (within-run precision)
  within-laboratory precision (Total precision)
  Lot to lot precision
  3 Lots of **AFIAS CK-MB** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
CK-MB [ng/mL]	Repeatability		Total precision		Lot to lot precision	
	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)
6.3	6.31	6.44	6.30	6.90	6.30	6.47
12.5	12.65	6.89	12.49	6.85	12.51	6.44
50.0	49.77	6.55	49.95	6.45	50.11	6.51

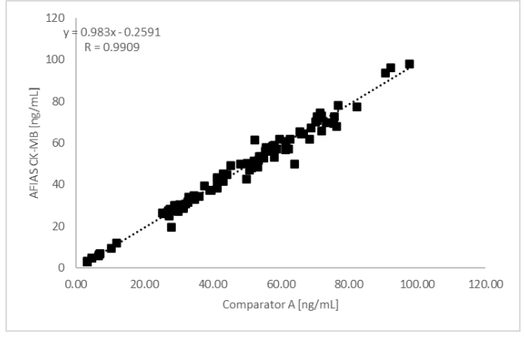
- Multi-site study
  Reproducibility
  1 Lot of **AFIAS CK-MB** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Multi-site study		
CK-MB [ng/m	Reproducibility	
	AVG [ng/mL]	CV (%)
6.3	6.3	6.48
12.5	12.4	3.10
50.0	50.3	6.35

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

Expected value [ng/mL]	LOT 1	LOT 2	LOT 3	AVG [ng/mL]	Recovery (%)
50.00	50.32	50.70	50.74	50.58	101.2
41.26	41.45	40.52	41.42	41.13	99.7
32.52	32.62	32.24	32.33	32.40	99.6
23.78	23.88	23.60	23.96	23.81	100.1
15.04	15.17	15.03	15.33	15.18	100.9
6.30	6.09	6.26	6.25	6.20	98.4

- **Comparability**
  CK-MB concentration of 100 clinical samples were independently with **AFIAS CK-MB (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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