Cardiac

AFIAS Cardiac Triple

INTENDED USE

AFIAS Cardiac Triple is a fluorescence immunoassay (FIA) for the quantitative determination of **cardiac Tn-I (Troponin-I), CK-MB (Creatine kinase) and Myoglobin** in human whole blood/serum/plasma Troponin-I, CK-MB, and Myoglobin value are used to assist in the diagnosis of acute myocardial infarction (AMI) and acute coronary syndrome (ACS).

For *in vitro* diagnostic use.

INTRODUCTION

Blood protein markers play an important role in the diagnosis of AMI. Tn-I, CK-MB, and Myoglobin are key members of them.

Cardiac troponins are currently the most sensitive and specific biochemical markers of myocardial necrosis. There are three types of troponin in heart muscle fibers: troponin-C, troponin-I, and troponin-T. Together they contribute to make cardiac muscle fibers contract. The clinical measurement of serum Tn-I has become an important tool in the diagnosis of the acute myocardial infarction. Serum Tn-I is more reliable than creatine kinase as a prognostic marker in people with ischemic chest pain. National and international scientific organizations have suggested the use of troponins, Tn-I and Tn-T, when implementing new diagnostic strategies in patients with acute coronary syndrome.

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 injuries 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 are 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 7.0ng/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.

Myoglobin is an iron- and oxygen-binding protein found in both skeletal and myocardial muscles. It acts as a transport protein and is involved in diffusion of oxygen in the muscle tissue. Myoglobin is a single-chain globular protein of 154 amino acids. It is composed of a central iron-containing 'Heme' which is enclosed in a compact bundle-like or prism-like arrangement formed by the eight right-handed α-helices[1,2]. Being a cytoplasmic protein having low molecular weight (of 17,699 Daltons), myoglobin is released into the serum more rapidly as compared to other cardiac markers upon damage to the myocardial cells. Serum concentration of myoglobin increases above the normal range as early as 1 hour after acute myocardial infarction (AMI), attains peak level in approximately 4 to 8 hours after the onset and normalize rapidly afterwards. Thus, myoglobin is better suited as a cardiac marker for early diagnosis of AMI. However, the elevated myoglobin is not specific to AMI owing to its large quantities in skeletal muscles as well. Despite its low clinical specificity and weak predictive value towards AMI, myoglobin is still a promising cardiac marker when other markers such as Creatin Kinase Isoenzyme-MB (CK-MB) and Cardiac Troponin-I (cTn-I) as well as other indicators like clinical signs and ECG are taken into account for diagnosis/confirmation of AMI[3-8]

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 Tn-I/CK-MB/Myoglobin concentration in the sample.

COMPONENTS

AFIAS Cardiac Triple 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 human CK-MB at the test line 1, anti-human Myoglobin at the test line 2, streptavidin at the test line 3, and chicken IgY at the control line.
- The detector part contains anti-Tn-I-fluorescence conjugate, biotin-anti-Tn-I conjugate, anti-chicken IgY-fluorescence conjugate, sucrose and sodium azide as a preservative in Tris-Cl buffer.
- The diluent part contains anti human CK-MB fluorescence conjugate, anti-human Myoglobin-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate and sodium azide as a preservative in Tris-Cl buffer.

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 they 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 AFIAS Cardiac Triple when biotin concentration in the sample was below 2 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 Cardiac Triple** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS Cardiac Triple** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

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

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

Storage condition			
Component	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. Re-seal along entire edge of zip-seal.

MATERIALS SUPPLIED

REF SMFP-59

Components of **AFIAS Cardiac Triple**

- 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 from **AFIAS Cardiac Triple.**

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 Cardiac Triple Control** REF CFPO-204
- **Boditech Cardiac Triple Calibrator** REF CFPO-205

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS Cardiac Triple** 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.

TEST SETUP

- Check the components of the **AFIAS Cardiac Triple** as described below. : Cartridges, pipette tips, an ID chip, a spare cartridge zipper bag and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the ID chip.
- 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 test.
- 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 Tn-I, CK-MB and Myoglobin concentration of the test sample in terms of ng/mL.

Item	Tn-I [ng/mL]	CK-MB [ng/mL]	Myoglobin [ng/mL]
Reference range	≤0.04 (99th percentile)	≤7.00 (95th percentile)	≤70.00 (97.5th percentile)
Working range	0.01-15	3-100	5-500

- Expected Values
  - In studies performed with the **AFIAS Cardiac Triple** assay involving 100 healthy volunteers in Korea, the upper reference limit (99th percentile, 95th percentile, 97.5th percentile) for Tn-I was 0.04 ng/mL and CK-MB was 7 ng/ml and Myoglobin was 70 ng/ml.
  - Due to the release kinetics of Tn-I, CK-MB and Myoglobin, a result below the decision limit within the first hours of the onset of symptoms does not rule out myocardial infarction with certainty. If myocardial infarction is still suspected, repeat the test at appropriate intervals.

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 Cardiac Triple. 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**

	Tn-I (ng/mL)	CK-MB (ng/mL)	Myoglobin (ng/mL)
Limit of Blank	0.008	0.67	1.25
Limit of Detection	0.01	1.31	1.64
Limit of Quantitation	0.03	3.00	5.00

- **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 Cardiac Triple test results did not show any significant cross-reactivity with these biomolecules.

Tn-I	
Cross reactants	Concentration
CK-MB	1,000 ng/mL
D-Dimer	20,000 ng/mL
NT-proBNP	1,000 ng/mL
Myoglobin	2,000 ng/mL
CK-MB	
Cross reactants	Concentration
Troponin complex	1,000 ng/mL
D-Dimer	20,000 ng/mL
NT-proBNP	1,000 ng/mL
Myoglobin	2,000 ng/mL
Myoglobin	
Cross reactants	Concentration
Troponin complex	1,000 ng/mL
CK-MB	1,000 ng/mL
D-Dimer	20,000 ng/mL
NT-proBNP	1,000 ng/mL

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

Interferents	Concentration
L-Ascorbic acid	350 µmol/L
Bilirubin	350 µmol/L
Cholesterol	13 mmol/L
D-Glucose	1,000 mg/dL
Hemoglobin	2 g/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 Cardiac Triple** were tested for 21 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Tn-I [ng/mL]	Repeatability		Total precision (within-laboratory precision)	
	AVG	CV (%)	AVG	CV (%)
0.23	0.23	5.53	0.23	5.30
0.94	0.94	5.93	0.94	6.14
7.50	7.40	6.05	7.47	5.91

Tn-I	Lot to lot precision	
[ng/mL]	AVG	CV (%)
0.23	0.23	6.29
0.94	0.93	5.86
7.50	7.48	5.89

CK-MB [ng/mL]	Repeatability		Total precision (within-laboratory precision)	
	AVG	CV (%)	AVG	CV (%)
6.30	6.21	5.14	6.19	5.11
12.50	12.35	5.58	12.44	5.76
50.00	49.85	5.11	49.90	5.23

CK-MB	Lot to lot precision	
[ng/mL]	AVG	CV (%)
6.30	6.24	5.40
12.50	12.48	5.63
50.00	49.64	5.92

Myoglobin [ng/mL]	Repeatability		Total precision (within-laboratory precision)	
	AVG	CV (%)	AVG	CV (%)
12.50	12.61	5.19	12.57	5.04
52.00	51.34	5.50	51.73	5.50
180.00	182.66	5.51	180.75	5.92

Myoglobin	Lot to lot precision	
[ng/mL]	AVG	CV (%)
12.50	12.54	5.32
52.00	52.17	6.10
180.00	181.14	5.84

  - Multi-site study
    Reproducibility
    1 Lot of **AFIAS Cardiac Triple** 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			
Tn-I	Reproducibility		
[ng/mL]	Mean [ng/mL]	SD	CV (%)
0.23	0.23	0.01	5.89
0.94	0.94	0.05	5.63
7.50	7.42	0.43	5.76

Multi-site study			
CK-MB	Reproducibility		
[ng/mL]	Mean [ng/mL]	SD	CV (%)
6.30	6.31	0.37	5.94
12.50	12.68	0.64	5.04
50.00	50.17	2.97	5.92

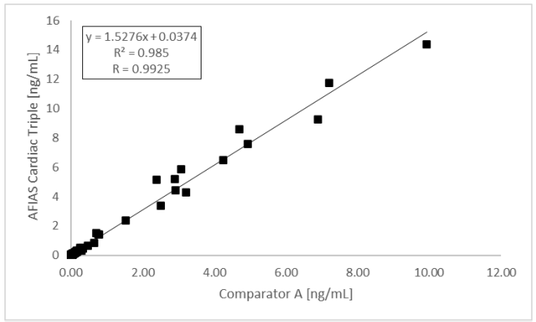
Multi-site study			
Myoglobin	Reproducibility		
[ng/mL]	Mean [ng/mL]	SD	CV (%)
12.50	12.41	0.71	5.73
52.00	52.16	3.20	6.14
180.00	178.80	10.19	5.70

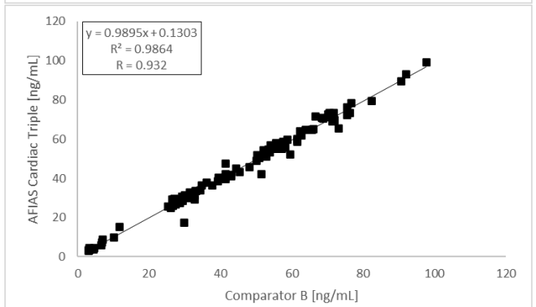
- **Accuracy**
  The accuracy was confirmed by testing with 3 different lots of AFIAS Cardiac Triple. The tests were repeated 10 times at each concentration of the control standard.

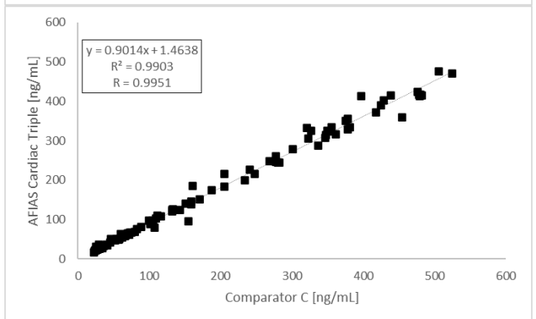
Tn-I [ng/mL]	Lot 1	Lot 2	Lot 3	Mean	Recovery (%)
0.23	0.23	0.23	0.23	0.23	100.3
0.94	0.91	0.92	0.95	0.93	98.4
7.5	7.61	7.38	7.37	7.45	99.4
CK-MB [ng/mL]	Lot 1	Lot 2	Lot 3	Mean	Recovery (%)
6.3	6.32	6.37	6.34	6.34	100.6
12.5	12.23	12.54	12.44	12.4	99.2
50	49.68	50.59	50	50.09	100.2
Myoglobin [ng/mL]	Lot 1	Lot 2	Lot 3	Mean	Recovery (%)
12.5	12.48	12.8	12.66	12.64	101.2
52	51.81	51.5	52.08	51.8	99.6
180	171.98	181.31	177.4	176.89	98.3

- **Comparability**
  Tn-I concentration of 100 clinical samples were quantified independently with **AFIAS Cardiac Triple (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 follow. CK-MB concentrations of 100 clinical samples were quantified independently with **AFIAS Cardiac Triple** and **comparator B** 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 follow. Myoglobin concentrations of 100 clinical samples were quantified independently with **AFIAS Cardiac Triple** and **comparator C** 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 follow.







REFERENCES

1. C. Daniel Cabaniss, Creatine Kinase, in: H.K. Walker, W.D. Hall, J.W. Hurst (Eds.), Clinical Methods: The History, Physical, and Laboratory Examinations, 3rd Ed.,Butterworths, Boston, 1990, pp 161-163.
2. Adams, J.E., Abendschein, D.R., Jaffe A.S., Biochemical markers of myocardial injury: Is MB creatine kinase the choice for the 1990s, Circulation, 1993; 88: 750-63.
3. Kent Lewandrowski, Ahchean Chen and James Januzzi, Cardiac markers for myocardial infarction, Am J Clin Pathol 2002;118 (Suppl 1):S93-S99.
4. Analysis of creatine kinase, CK-MB, myoglobin, and troponin T time- activity curves for early assessment of coronary artery reperfusion after intravenous thrombolysis Circulation. 1993;87:1542-1550.
5. Simultaneous Rapid Measurement of Whole Blood Myoglobin, Creatine Kinase MB, and Cardiac Troponin I by the Triage Cardiac Panel for detection of Myocardial Infarction Clinical Chemistry 45:2 199–205 (1999).
6. Diagnostic Marker Cooperative Study for the Diagnosis of myocardial Infarction Circulation. 1999;99:1671-1677
7. Bedside Multimarker Testing for Risk Stratification in Chest Pain Units: The Chest Pain Evaluation by Creatine Kinase-MB, Myoglobin, and Troponin I (CHECKMATE) Study Circulation. 2001;103:1832-1837
8. Mauro Panteghini, Franca Pacani, Kiang-Teck J.Yeo, Fred S. Apple, Robert H. Christenson. Francesco Dati, Johannes Mair, Jan Ravkilde, and Alan H.B. We. Evaluation of Imprecision for Cardiac Troponin Assays at Low-Range Concentrations. 2004;50:2:327-332.
9. Alan McNeil, PhD, FRACP, FRCPA. The Trouble with Troponin. Heart, Lung and Circulation 2007;16;S13-S16.
10. David M. Bunk and Micahel J. Welch. Characterization of a New Certified Reference Material for Human Cardiac Troponin I. Clinical Chemistry 2002;52:2:212-219
11. Jaffe AS, Ravkilde J, Roberts R, Naslund U, Apple FS, Galvani M, Katus H. It's time for a change to a troponin standard. Circulation 2000;102:1216 –1220.
12. Jillan R. Tate, David Heathcote, Gus Koerbin, Gary Thean, David Andriske, Jone Bonar, Janice Gill. Theharmonization of cardiac troponin I measurement is independent of sample time collection but is dependent on the source of calibrator. Clinica Chimica Acta 324:2002:13-23.
13. Ohman EM, Armstrong PW, Christenson RH, et al. Cardiac troponin T levels for risk stratification in acute myocardial ischemia. N Engl J Med 1996;335:1333– 41.
14. Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiacspecific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. N Engl J Med 1996;335:1342 –9.
15. Cox, MM, Nelson, DL. Lehninger: Principles of Biochemistry, 3rd edition. W.H. Freeman and Company, New York, 2000, 206.
16. Ordway GA, Garry DJ. Myoglobin: An essential hemoprotein in striated muscle. J Exp Biol.. 2004;207(Pt 20):3441-6.
17. Vaidya HC. Myoglobin: an early biochemical marker for the diagnosis of acute myocardial infarction. J Clin Immunoassay. 1994;17:35-39.
18. Gibler WB, Gibler CD, Weinshenker C, et al. Myoglobin as an early indicator of acute myocardial infarction. Ann Emerg Med. 1987;16:851-856.
19. Mair J, Morandell D, Genser N, et al. Equivalent early sensitivities of myoglobin, creatine kinase–MB mass, creatine kinase isoforms ratios, and cardiac troponins I and T for acute myocardial infarction. Clin Chem. 1995;41:1266-1272.
20. Mercer DW. Role of cardiac markers in evaluation of suspected myocardial infarction. Postgrad Med. 1997;102:113-122

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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