Getein Biotech, Inc.

CE

CK-MB/cTnI/Myo Fast Test Kit (Immunofluorescence Assay) For *in vitro* Diagnostic Use

User Manual

INTENDED USE

CK-MB/cTnI/Myo Fast Test Kit (Immunofluorescence Assay) is intended for *in vitro* quantitative determination of CK-MB/cTnI/Myo in serum, plasma or whole blood. This test is used as an aid in the clinical diagnosis, prognosis and evaluation of myocardial injury such as Acute Myocardial Infarction (AMI), Unstable Angina, Acute Myocarditis and Acute Coronary Syndrome (ACS).

SUMMARY

Creatine kinases are dimer isozymes composed of two monomer subunits, CK-M (for skeletal muscle derived) and CK-B (for brain derived), which can form all three combinations of monomers: CK-BB, CK-MM, and CK-MB. BB is found primarily in the brain. Skeletal muscles primarily contain the MM isoform, with trace amount of MB (around 1-4% of total CK activity). Cardiac muscles also contain the MM isoform, but higher amount of MB, typically around 20% of total CK activity. CK-MB is a more sensitive marker of myocardial injury than total CK activity, because it has a lower basal level and a much narrower normal range. Medical literatures commonly state that CK-MB levels are elevated in 4 to 6 hours, peak at 10 to 24 hours, and return to normal within 3 to 4 days after an acute myocardial infarction. Classically, an increase of the myocardial-specific enzyme CK-MB is considered as the hallmark of acute myocardial infarction, and increased levels are frequently interpreted by the clinician as objective evidence of myocardial cell damage.

Troponin complex consists of three regulatory proteins: T, which connects the troponin complex and tropomyosin (another cardiac muscle regulatory protein); I, which prevents muscle contraction in the absence of calcium; and C, which binds calcium. Cardiac troponin I (MW 22.5 kDa) and the two skeletal muscle isoforms of troponin I have considerable amino acid sequence homology, but cTnI contains an additional N-terminal sequence and is highly specific for myocardia.

Clinical studies have demonstrated the release of cTnI into the blood stream within hours following acute myocardial infarctions (AMI) or ischemic damage. Elevated levels of cTnI are detectable in blood within 4 to 6 hours after the onset of chest pain, reaching peak concentrations in approximately 8 to 28 hours, and remain elevated for 3 to 10 days following AMI. Due to the high myocardial specificity and the long duration of elevation, cTnI has become an important marker in the diagnosis and evaluation of patients suspected of having an AMI.

Myoglobin is a small monomeric protein which serves as an intracellular oxygen storage site. It is found in abundance in the muscle and can get through into the blood circulation directly when myocardial cell is damaged mildly. Therefore, myoglobin has been advocated as a sensitive marker for early acute myocardial injury by American College of Cardiology Committee.

PRINCIPLE

Mixed monoclonal antibodies against human CK-MB, cTnI and Myo were conjugated with fluorescence latex and another set of anti-human CK-MB/cTnI/Myo monoclonal antibodies were coated on different test lines respectively. After the sample has been applied to the test strip, the fluorescence latex-labelled anti-human CK-MB, cTnI and Myo monoclonal antibodies will bind with the CK-MB, cTnI and Myo in sample respectively and form marked antigen-antibody complexes. These complexes move to the test card detection zone by capillary action. Then marked antigen-antibody complexes will be captured on different test lines by another set of monoclonal antibodies against human CK-MB, cTnI or Myo respectively resulting in the accumulation of fluorescence particles on the test lines. The fluorescence intensity of each test line increases in proportion to the amount of CK-MB, cTnI or Myo in sample.

Then insert test card into Getein1100 Immunofluorescence Quantitative Analyzer (hereafter referred to as Getein1100), the concentration of CK-MB, cTnI and Myo in sample will be determined and displayed on the screen. The value will be stored in Getein1100 and available for downloading. The result can be easily transmitted to the laboratory or hospital information system.

CONTENTS

A kit contains:

1. Getein CK-MB/cTnI/Myo test card in a sealed pouch with desiccant…25
2. Disposable pipet……25
3. User manual ……1
4. SD card ……1
5. Blood sample diluent……1

A test card consists of:

A plastic shell and a reagent strip which is composed of a sample pad, nitrocellulose membrane (one end of the membrane is coated with a fluorescence latex-labelled anti-human CK-MB, cTnI and Myo monoclonal antibodies, the test line T_1 is coated with another anti-human CK-MB monoclonal antibody, T_2 is coated with another anti-human cTnI monoclonal antibody, T_3 is coated with another anti-human Myo monoclonal antibody, and the control line C is coated with rabbit anti-mouse IgG antibody), absorbent paper and liner.

Blood sample diluent:

Phosphate buffered saline, proteins, detergent, preservative, stabilizer.

Note: Components from different batches must not be interchanged.

APPLICABLE DEVICE

Getein1100 Immunofluorescence Quantitative Analyzer

STORAGE AND STABILITY

Store the test card at 4~30ºC with a valid period of 24 months.
Use the test card within 1 hour once the foil pouch is opened.
Store the blood sample diluent at 0~30ºC with a valid period of 24 months.
Store the blood sample diluent at 2~8ºC for better results.

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. For professional use only.
3. Do not use the kit beyond the expiration date.
4. Do not use the test card if the foil pouch is damaged.
5. Do not open pouches until ready to perform the test.
6. Do not reuse the test card.
7. Do not reuse the pipet.
8. Handle all specimens as potentially infectious. Proper handling and disposal methods should be followed in accordance with local regulations.
9. Carefully read and follow the manual to ensure proper test performance.

SPECIMEN COLLECTION AND PREPARATION

1. This test can be used for serum, plasma and whole blood samples. Heparin should be used as the anticoagulant for plasma and whole blood. Samples should be free of hemolysis.
2. Suggest to use serum or plasma for better results.
3. Serum or plasma can be used directly. For whole blood sample, one drop of blood sample diluent must be added before testing.
4. If testing will be delayed, serum and plasma samples may be stored up to 7 days at 2~8 ºC or stored at -20 ºC for 6 months prior to being tested (whole blood sample may be stored up to 3 days at 2~8 ºC).
5. Refrigerated or frozen sample should reach room temperature and be homogeneous prior to testing. Avoid multiple freeze-thaw cycles.
6. Do not use heat-inactivated samples.
7. SAMPLE VOLUME: 100 µl.

TEST PROCEDURE

1. Collect specimen according to manual.
2. Test card, sample and reagent should be brought to room temperature prior to testing.
3. Confirm SD card lot No. in accordance with test kit lot No.. Perform “SD Card Calib” calibration when necessary (Details refer to 8.5.2 of Getein1100 User Manual).
4. On the main interface of Getein1100, press "ENT" button to enter testing interface.
5. Remove the test card from the sealed pouch immediately before use. Label the test card with patient or control identification.
6. Put the test card on a clean table, horizontally placed.
7. Using sample transfer pipette, deliver 100 µl of sample (or 3-4 drops of

sample when using disposable pipet) into the sample port on the test card (for whole blood sample, one drop of blood sample diluent must be added after loading 100 µl sample on the test card).

8. After 15 minutes, insert the test card into Getein1100 and press "ENT" button. The result will be shown on the screen and printed automatically.

Notes:

1. It is required to perform “SD Card Calib” calibration when using a new batch of kit.
2. It is suggested to calibrate once for one batch of kit.
3. Make sure the test card insertion is correct and complete.

TEST RESULTS

Getein1100 can scan the test card automatically and display the result on the screen. Please follow the procedure in user manual of Getein1100 for result printing.

For additional information, please refer to the user manual of Getein1100.

EXPECTED VALUE

The expected normal value for CK-MB was determined by testing samples from 500 apparently healthy individuals. The 99th percentile of the concentration for CK-MB is 5.0 ng/ml. CK-MB concentration less than 5.0 ng/ml can be estimated as normal.

The expected normal value for cTnI was determined by testing samples from 500 apparently healthy individuals. The 99th percentile of the concentration for cTnI is 0.1 ng/ml. cTnI concentration less than 0.1 ng/ml can be estimated as normal.

The expected normal value for Myo was determined by testing samples from 500 apparently healthy individuals. The 95th percentile of the concentration for Myo is 50 ng/ml. Myo concentration less than 50 ng/ml can be estimated as normal. The 97.5th percentile of the concentration for Myo is 70 ng/ml. Myo concentration less than 70 ng/ml can be estimated as normal.

It is recommended that each laboratory establish its own expected values for the population it serves.

PERFORMANCE CHARACTERISTICS

Measuring Range:
CK-MB 2.5~80.0 ng/ml
cTnI 0.1~50.0 ng/ml
Myo 30~600.0 ng/ml
Lower Detection Limit：
CK-MB: ≤ 2.5 ng/ml
cTnI:≤ 0.1 ng/ml
Myo: ≤ 30 ng/ml
Within-Run Precision: ≤10%
Between-Run Precision: ≤15%

LIMITATIONS

1. As with all diagnostic tests, a definitive clinical diagnosis should not be made based on the result of a single test. The test results should be interpreted considering all other test results and clinical information such as clinical signs and symptoms.

2. Samples containing interferents may influence the results. The table below listed the maximum allowance of these potential interferents.

Interferent	Concentration (Max)
Hemoglobin	5 g/L
Triglyceride	10 g/L
Bilirubin	0.2 g/L

REFERENCES

1. Mauro Pantaghini; Undefined International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Scientific Division Committee on Standardization of Markers of Cardiac Damage. Clin Chem Lab Med, 1998, 36:887~893.
2. Antman EM, Anbe DT, Armstrong PW, et al. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the Manage 2004).
3. EN ISO 18113-1:2009 In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 1: Terms, definitions and general requirements.
4. EN ISO 18113-2:2009 In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: In vitro diagnostic reagents for professional use (ISO 18113-2:2009).

DESCRIPTION OF SYMBOLS USED

The following graphical symbols used in or found on CK-MB/cTnI/Myo Fast Test Kit (Immunofluorescence Assay) are the most common ones appearing on medical devices and their packaging. They are explained in more detail in the European Standard EN 980: 2008 and International Standard ISO 15223-1:2007.

Key to symbols used			
	Manufacturer		Expiration Date
	Do not re-use		Date of manufacture
	Consult instructions for use	LOT	Batch code
	Temperature limitation	IVD	In vitro diagnostic medical device
	Sufficient for	EC REP	Authorized representative in the European Community
CE	CE marking		

Thank you for purchasing CK-MB/cTnI/Myo Fast Test Kit (Immunofluorescence Assay).
Please read this manual carefully before operating to ensure proper use.

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