MAGLUMI CK-MB (CLIA)

REF 130206001M Σ 100

EC REP

Shenzhen New Industries Biomedical Engineering Co., Ltd
4F,Wearnes Tech Bldg,
Science & Industry Park,
Nanshan,Shenzhen,518057CHINA
Tel. + 86-755-86028224
Fax.+ 86-755-26654850

Lotus Global Co., Ltd
15 Alexandra Road
London
NW8 0DP
UK
Tel. + 44-20-75868010
Fax.+ 44-20-79006187

CE

FOR PROFESSIONAL USE ONLY
Store at 2...8 °C

COMPLETELY READ THE INSTRUCTIONS BEFORE PROCEEDING

en

SYMBOLS EXPLANATIONS

EC REP	Authorized Representative in Europe
	Manufacturer
	Attention. See Instructions For Use
CONT	Contents of kit
IVD	In vitro diagnostic medical device (In vitro diagnostic use)
LOT	Lot number
REF	Catalogue Code
	Expiry date (Use by…)
8°C 2°C	Temperature limitation (store at 2...8 °C)
Σ	Number of tests
	Keep away from sunlight
	Keep upright

INTENDED USE

The kit has been designed for the quantitative determination of the MB isoenzyme of creatine kinase (CK-MB) in human serum.
The method can be used for samples over the range of 0-300 ng/ml.
The test has to be performed on the MAGLUMI chemiluminescence immunoassay (CLIA) fully auto analyzer (Including MAGLUMI 1000, MAGLUMI 2000, MAGLUMI 2000 Plus and new developed models).

SUMMARY AND EXPLANATION OF THE TEST

Creatine Kinase-MB (CK-MB) is the enzyme being used as the definitive serum marker for the diagnosis or exclusion of acute myocardial infarction (AMI).
The determination of CK-MB mass has proven to be more specific for myocardial necrosis than the long-standing CK-MB activity and CK-MB inhibition assays.
CK-MB, released after AMI, is detectable in blood as early as 3-4 hours after the onset of symptoms, and remains elevated for approximately 65 hours post infarct. CK-MB mass levels are reportedly 50% diagnostic for AMI after 3 hours and >90% diagnostic at 6 hours 10.Such accuracy makes CK-MB mass determinations useful in confirming AMI in patients presenting to the ER with non-diagnostic ECGs > 6 hours after the onset of symptoms.

PRINCIPLE OF THE TEST

Sandwich immunoluminometric assay;
Use an anti-CK-MB monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator or Control with ABEI Label, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37℃, forming a sandwich; after sediment in a magnetic field, decant the supernatant, then cycle washing for 3 times. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of CK-MB present in controls or samples.

CONT KIT COMPONENTS

Material Supplies

Reagent Integral for 100 determinations	
Nano magnetic microbeads: TRIS buffer, 1.2% (W/V), 0.2%NaN_3, coated with sheep anti- FITC polyclonal antibody.	2.5ml
Calibrator Low: bovine serum, 0.2%NaN_3	2.5ml
Calibrator High: bovine serum, 0.2%NaN_3	2.5ml
FITC Label: anti-CK-MB monoclonal antibody labeled FITC, contains BSA, 0.2%NaN_3.	6.5ml
ABEI Label: anti-CK-MB monoclonal antibody labeled ABEI, contains BSA, 0.2%NaN_3.	6.5ml
All reagents are provided ready-to-use.	

Reagent Vials in kit box	
Internal Quality Control: containing BSA, 0.2%NaN_3. (target value refer to Quality Control Information date sheet)	2.0ml

Accessories Required But Not Provided

MAGLUMI Reaction Module	REF: 630003
MAGLUMI Starter 1+2	REF: 130299004M

MAGLUMI Wash Concentrate	REF: 130299005M
MAGLUMI Light Check	REF: 130299006M

Preparation of the Reagent Integral

Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!) Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange integral component from different reagents or lots!

Storage and Stability

- Sealed: Stored at 2-8 ℃ until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's not going to be used on board during the next 12 hours.
-  Keep upright for storage.
-  Keep away from direct sunlight.

CALIBRATION AND TRACEABILITY

1)Traceability

Calibrators in the Reagent Kit are from Fitzgerald

To perform an accurate calibration, we have provided the test calibrators standardized against the SNIBE internal reference substance.

2) 2-Point Recalibration

Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

3) Frequency of Recalibration

- After each exchange of lot (Reagent Integral or Starter Reagents).
- Every 2 week and/or each time a new Integral is used (recommendation).
- After each servicing of the MAGLUMI Fully Auto analyzer.
- If controls are beyond the expected range.

SPECIMEN COLLECTION AND PREPARATION

Sample material: serum

Collect samples using standard procedures.

Store at 2-8 ℃: 24 hours, for longer storage periods, freeze to below - 20 ℃

Avoid repeated freezing and thawing cycles, stored samples should be thoroughly mixed prior to use (Vortex mixer).

Please ask local representative of SNIBE for more details if you have any doubt.

Vacuum Tubes

(a) Blank tubes are recommended type for collecting samples.

(b) Please ask SNIBE for advice if special additive must be used in sample collecting.

Specimen Conditions

- Do not use specimens with the following conditions:
  (a) heat-inactivated specimens;
  (b) Cadaver specimens or body fluids other than human serum;
  (c) Obvious microbial contamination.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed **thoroughly** after thawing by **low** speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens or controls) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service, for a more detailed discussion of onboard sample storage constraints.

Storage

- If testing will be delayed for more than 8 hours, remove serum or plasma from the serum or plasma separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored up to 24 hours at 2-8°C.
- Specimens can be stored up to 30 days frozen at -20°C or colder.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the serum or plasma separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

WARNING AND PRECAUTIONS FOR USERS

- For use in *IN-VITRO* diagnostic procedures only.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

CAUTION: This product requires the handling of human specimens.

- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent; these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of

the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach (USP 24,2000, p.2143). A minimum of one hour at 121 ℃ is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.

- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens13. Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
- Safety data sheets are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the kit surface, please pay attention the silicon film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the MAGLUMI Fully Auto analyzer. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the MAGLUMI Chemiluminescence Analyzer Operating Instructions.

40μl	Sample, calibrator or controls
+40μl	ABEI Label
+40μl	FITC Label
+20μl	Nano magnetic microbeads
15 min	Incubation
400μl	Cycle washing
3 s	Measurement

DILUTION

Sample dilution by analyzer is not available in this reagent kit.

Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor.

Please choose applicable diluents or ask SNIBE for advice before manual dilution must be processed.

QUALITY CONTROL

- Observe quality control guidelines for medical laboratories
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

LIMITATIONS OF THE PROCEDURE

1) Limitations

A skillful technique and strict adherence to the instructions are necessary to obtain reliable results. Bacterial contamination of samples or repeated freeze-thaw cycles may affect the test results. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin<0.06mg/ml, haemoglobin<16mg/dl or triglycerides< 12.5mg/ml.

3) HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralising agents are added, extremely high HAMA serum concentrations may occasionally influence results.

4) High-Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the MAGLUMI CK-MB assay, no high dose hook effect was observed when samples containing up to 5000 ng/ml.

RESULTS

1) Calculation of Results

- The analyzer automatically calculates the CK-MB concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/ml. For further information please refer to the MAGLUMI Chemiluminescence Analyzer Operating Instructions.
-

2) Interpretation of Results

- Results of study in clinical centers with group of individuals, 95% of the results were：< 5 ng/ml
- Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1) Precision

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

Intra-assay precision			
Control	Mean(ng/ml)	SD(ng/ml)	CV%
Level 1	6.03	0.55	4.21%
Level 2	22.66	0.92	4.07%
Level 3	90.05	3.70	4.11%

Inter-assay coefficient of variation was evaluated on three batches of kit. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

Inter-assay precision			
Control	Mean(ng/ml)	SD(ng/ml)	CV%
Level 1	6.74	0.50	7.46%
Level 2	21.49	1.55	7.23%
Level 3	91.03	6.54	7.18%

2) Analytical Sensitivity

The sensitivity is defined as the concentration of CK-MB equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 0.3 ng/ml.

3) Specificity

The specificity of the CK-MB assay system was assessed by

measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross reactivity
CK-MM	500 ng/ml	0.9%

4) Recovery

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluents, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90% -110%.

Expected	Mean Measuring	Recovery
128.9 ng/ml	135.2 ng/ml	105%

5) Linearity

Use CK-MB calibrator to prepare the six-point standard curve, measuring all points' RLU except point A, and then do four-parameter linear fitting in double logarithm coordinate, the absolute linear correlation coefficient(r) should be bigger than 0.9800.

Calibrator Point	Concentration ng/ml	Absolute linear correlation coefficient (r)
A	0	
B	2.5	r=0.9870
C	10	
D	25	
E	100	
F	500	

6) Method comparison

A comparison of MAGLUMI CK-MB (y) with a commercially available CK-MB test (x) using clinical samples gave the following correlations (ng/ml):

Linear regression
y = 1.10x-6.3
r = 0.959
Sy.x =11.3

Number of samples measured: 180
The sample concentrations were between 1.3 and 440 ng/ml.

REFERENCES

1. Wu, A. (2006). Tietz Clinical Guide to Laboratory Tests, Fourth Edition. Saunders Elsevier, St. Louis, Missouri. Pp. 312-315.
2. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. Burtis CA, Ashwood ER and Bruns DE, eds. 4th ed. St. Louis, Missouri: Elsevier Saunders; 2006. P. 599.
3. Engel G, Rockson SG. Rapid diagnosis of myocardial injury with troponin T and CK-MB relative index. Mol Diagn Ther. 2007;11(2): 109-16.
4. Ernst-Georg Krause,Georg Rabitzsch,Franz Noll,Johannes Maire,Bernd Puschendorf. Glycogen phosphorylase isoenzyme BB in diagnosis of myocardial ischaemic injury and infarction [J] ,1996.
5. Karras DJ,Kane DL.Serum markers in the emergency department di-agnosis of acute myocardial infarction[J]. Emergency Medicine Clinics of North America, 2001.
6. Ohman E M,Armstrong P W,Christenson R H,et al.Cardiac troponin T levels for risk stratification in acute myocardial ischemia.GUSTO IIA Investigators. The New England Journal of Medicine, 1996.
7. Miida T,Oda H,Toeda T,et al.Additional ST-segment elevation immediately after reperfusion and its effect on myocardial salvage in anterior wall acute myocardial infarction. The American Journal of Cardiology, 1994
8. Kosuge M,Kimura K,Nemoto,et al.Clinical significance of late peak formation of creatine kinase in patients with acute anterior myocardial infarction after successful reperfusion. Journal of Cardiology, 1995.
9. Shell W,Mickle DK.Swan HJC: Effects of nonsurgical myocardial reperfusion on plasma creatine kinase kinetics in man. American Heart Journal, 1983.
10. Blanke H,Von Hardenberg D,Cohen M,et al.Patterns of creation kinase release during acute myocardial infarction after nonsurgical reperfusion: comparison with conventional treatment and correlation with infarct size. Journalism Assn of Community College, 1983.
11. Schwarz F,Schule G,Karsch KR,et al.Intracoronary thrombolysis in acute myocardial infarction: correlation among serum enzyme, scintigraphic and hemodynamic findings. The American Journal of Cardiology, 1982.
12. Shell WE,Swan HJC.Ganz W: Accelated washout of creatine kinase by fibrolytic reperfusion in man (abstr). Clinical Research, 1981.
13. Nayler WG.Elz JS: Reperfusion injury: laboratory artifact or clinical dilemma. Circulation, 1986.
14. Maroko PR,Libby P,Covell JW,et al.Precordial ST segment elevation mapping: An artraumatic method for assessing alterations in the extent of myocardial ischemic injury. The American Journal of Cardiology, 1972.
15. Muller JE,Maroko PR.Braunwald E: Precordial eletrocardiographic mapping: A technique to assess the efficacy of interventions designed to limit infarct size. Circulation, 1978.