UMA CO., LTD.

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Matsudo, Chiba, Japan

MEASURE CKMB

Reagent for determination of Creatine Kinase Isozyme

IFCC Method

2 - 8°C	**IVD** *In vitro* Diagnostics	**QUALITY MANAGEMENT SYSTEM (BY TUV)**
DO NOT freeze	18 months/block from light	**ISO 13485:2016**

1. PURPOSE OF USE

Providing a quantitative in vitro assay for the Creatine Kinase Isozyme (CK-MB) concentration in serum or plasma.

2. GENERAL INSTRUCTION

a. For in vitro diagnostics use only.

b. Diagnosis should be made in a comprehensive manner, in accordance with other related test results and clinical symptoms by the doctor in attendance.

c. For guaranteed results, usage of this product must comply with the instruction in this manual.

d. If you use automatic analyzers, follow their instructions carefully.

SUMMARY

Creatine Kinase MB Fraction (CK-MB) is a widely used biomarker for myocardial injury. CK-MB is found in relatively high concentration in the myocardium, but it is also present in skeletal muscle and other tissues (Al - Hadi and Fox, 2009). About 15 - 40% of total creatine kinase (CK) activity of the heart is due to CK-MB. The rapid rise of CK-MB after myocardial damage made it a particularly useful biomarker in the early diagnosis of AMI and re - infarction. CK-MB is released within 1 h following cardiac injury (Figure 1). In a study of 202 patients by Wu et al., CK-MB levels increased within 1 - 6 h after myocardial injury in up to 75% of patients (Wu et al., 1992). Within 7 - 12 h after myocardial injury, CK-MB levels are increased in 94% of patients. In another multicenter study by Zimmerman et al., CK-MB was the most sensitive and specific early marker for AMI (91% sensitivity and 89% specificity), followed by myoglobin (78% sensitivity and 89% specificity) (Zimmerman et al., 1999). In this study, CK-MB was also a reliable late biomarker of AMI, with sensitivity of 96% and specificity of 98%. While CK-MB is a valuable early biomarker of myocardial injury, it is less sensitive and specific than cardiac troponin, and because of this it has been replaced by troponin as the gold standard for diagnosis of AMI (Saenger and Jaffe, 2007).

3. MATERIALS REQUIRED BUT NOT INCLUDED

- Saline 0.9 % and high grade purified water

- Micropipet and other basic laboratory equipment.

- MEASURE Multi Calibrator and MEASURE Human Lyo L-1 and MEASURE Human Lyo L-2

4. REAGENT COMPOSITION & PREPARATION

- Reagent R-1: Hexokinase; Glucose-6-phosphate dehydrogenase (G6PDH); CK-MM antibody

  Reagent R-1 is ready for use

- Reagent R-2: Creatine phosphate

  Reagent R-2 is ready for use

- Once open, Reagent stored on board the instrument is stable for 30 days with Hitachi 7180 Analyzers.

- Applicable to various automated analyzers.

- Calibrator MEASURE Multi Calibrator (separately sold): Put 5 mL of purified water to the vials of Calibrator (MEASURE Multi Calibrator), leave at room temperature for 45 minutes and sometimes gently invert the vial before use. After reconstituting, Calibrator can be used without dilution.

- Controls MEASURE Human Lyo L-1 and MEASURE Human Lyo L-2 (separately sold): Put 5 mL of purified water to the vials of controls (Lyo L-1 and Lyo L-2); leave at room temperature for 45 minutes and sometimes gently invert the vial before use. After reconstituting, controls can be used without dilution.

5. SAMPLE PREPARATION & STORAGE

- Serum: Wait until the sample is completely coagulated. Take the supernatant to use as specimens.

- Plasma: Treat sample by anticoagulant: Li-Heparin, K2-EDTA plasma; leave sample to stand for 3 hours or centrifuge at 2000 rpm for 2 minutes; take the plasma layer (supernatant) and use as specimen.

- CK-MB in the sample is not stable at high temperature. Analyze samples soon after collection or store samples 2 – 8°C and analyze as soon as possible.

- Stability in serum

  - 8 hours at 20 - 25°C
  - 8 days at 2 - 8°C
  - 28 days at < -20°C

- Stability in heparin plasma

  - 8 hours at 20 - 25°C
  - 5 days at 2 - 8°C
  - 8 days at < -20°C

- Stability in EDTA plasma

  - 2 days at 20 - 25°C
  - 7 days at 2 - 8°C
  - 1 year at < -20°C

- See interferences section for details about possible sample interferences.

6. MEASUREMENT PRINCIPLE

Only CK-MB activity is retained in R-1. This CK-MB produces creatine and ATP using creatine phosphate and ADP as substrates. Next, ATP is oxidized to 6-phosphogluconate by ADP and glucose-6-phosphate dehydrogenase (G6PDH) by the action of hexokinase (HK) in the presence of glucose. Since $NADP^+$ is reduced to NADPH, CK-MB activity can be determined by measuring the increase rate of absorbance of NADPH.

1st reaction

$$CK + \text{CK-MM antibody} \longrightarrow \text{CK-MB}$$

$$\text{Phosphocreatine} + ADP \xrightarrow{CK-MB} \text{Creatine} + ATP$$

$$ATP + \text{Glucose} \xrightarrow{HK} ADP + \text{Glucose-6-phosphate}$$

2nd reaction

$$\text{Glucose-6-phosphate} + NADP \xrightarrow{G6PDH} \text{6-phosphogluconate} + NADPH + H^+$$

increase of absorbance at 340nm

7. ASSAY PROCEDURE

This product is compatible with various types of clinical analyzer. An example of the assay procedure is indicated below.

Sample	+	Reagent 1	$\xrightarrow[280s]{37^\circ C}$	Reagent 2
8.0µL		160µL		40µL
$\xrightarrow[180s]{37^\circ C}$		Meas. Abs I	$\xrightarrow[140s]{37^\circ C}$	Meas. Abs II
		(340/405nm)		(340/405nm)

$\longrightarrow$ ΔAbs at 340/405nm $\longrightarrow$ Acitivity of CK-MB

Perform the assay according to the instructions for operating the automated analyzer Hitachi models. Refer to the **13. INFORMATION FOR AUTOANALYZERS** for the details of the assay method. Contact **HUMA MEDICAL CO., LTD.** for information about the parameters for other automated analyzers.

8. CALCULATION & UNIT CONVERSION

Calculation

- Calculate ΔAbs of specimen & standards vs blank

- Plot a calibration curve CK-MB = f(ΔAbs)

- Calculate CK-MB in specimen using the curve

(doing same procedure for Controls)

Unit conversion

U/L x 0.0167 = µkat/L

9. PERFORMANCE & CORRELATION TEST

a. Measuring range

- The assay is linear within an CK-MB enzyme activity range of 1 - 2000 U/L.

- If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

b. Detection Limit

Limit of Blank (LoB)	=	0 U/L
Limit of Detection (LoD)	=	1 U/L
Limit of Quantitation (LoQ)	=	1 U/L

The LoB, LoD and LoQ were determined in accordance with CLSI EP17-A2 requirements.

The LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoB corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

The LoD is determined based on the LoB and standard deviation of low concentration samples. The LoD corresponds to the lowest analyte concentration which can be detected (value above the LoB with a probability of 95%).

The LoQ is the lowest analyte concentration that can be reproducibly measured with a total error of 20%. It has been determined using low concentration samples.

c. Performance

- Sensitivity: Using purified water, absorbance change is in 0.001 - 0.003 Abs/min, using controlled sample of 500 U/L, absorbance change is in 0.025 - 0.100 Abs/min.

- Accuracy: When measuring a control sample, the result is within ±10% of assigned value.

d. Precision (on Biolis 30i / SK300)

Representative performance data on the analyzers are given below.

Results obtained in individual laboratories may differ.

Precision was determined using controls followed the CLSI Approved Guidline EP5-A2 with repeatability, reproducibility and total precison (1 aliquot per run, 2 run per day, 20 days).The following results were obtained.

Criterion: CV of Repeatability (aka. Within-run precision) is less than 5% and Total Precision is less than 10%.

Repeatability	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
Control Lyo L-1	22.9	0.8	3.50
Control Lyo L-2	63.6	0.9	1.42

Reproducibility	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
Control Lyo L-1	22.9	0.77	3.36
Control Lyo L-2	63.6	1.74	2.74

Total precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
Control Lyo L-1	22.9	0.95	4.17
Control Lyo L-2	63.6	1.86	2.92

e. Correlation Test

Same principle method

Serum

Regression equation: y = 1.0106x - 0.7399 (n = 52)

Correlation coefficient: r = 0.9995

Plasma

Regression equation: y = 0.9942x - 0.5142 (n = 54)

Correlation coefficient: r = 0.9996

(y: value obtained from using UMA's reagent)

10. EXPECTED VALUES

Less than 25 U/L

Reference range should be established at each facility and judgement should be based on measurement results in a comprehensive manner together with clinical symptoms and other measurement results.

11. INTERFERENCES

- Icterus: No significant interference of conjugated bilirubin concentration up to 40 mg/dL and free bilirubin concentration up to 40 mg/dL

- Hemolysis: No significant interference of hemoglobin concentration in hemolyzed samples up to 15 mg/dL

- Lipemia (Intralipid): No significant interference triglycerides concentration up to 3000 FTU

- Ascorbic Acid: No significant interference of ascorbic acid concentration up to 50 mg/dL

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Please use another methods if the result is affected by any factors

12. HANDLING, USAGE & DISPOSAL

Handling

1. Specimen can be potentially positive for infectious agents including hepatitis B virus and HIV. Wear glove and goggle when needed.

2. In case reagents got into skin, eye or mouth by mistake, wash it immediately with plenty of water and consult the doctor if needed.

3. If reagents are spilled, dilute with water and wipe it out. If specimen is spilled, spray 80% of alcohol over the specimen and wipe it out.

Usage

1. Store reagents under specified condition. Do not use after expiration date.

2. Do not use the container and auxiliaries included in this kit for other purposes.

3. Do not mix reagents of different lot for use.

4. Do not add to the reagent being used even if it is the same lot number.

Disposal

1. All specimens, as well as all instruments (e.g. test tubes) that come in contact with the specimens, must be treated by the following methods, or they must be treated according to the manual for infectious medical waste provided in each facility.

- Sterilize with an autoclave, subjecting them to high pressure saturated steam at 121 °C for more than 20 minutes. Do not process waste containing sodium hypochlorite solution with an autoclave.

- Immerse at least one hour in sodium hypochlorite solution (active chloride concentration of over 1000 ppm).

2. This reagent contains sodium azide. Sodium azide can react with lead pipe and/or steel pipe and can generate explosive metal azide. Make sure to use plenty of water at disposal. Concentration of sodium azide in R-2 is 0.05%.

13. INFORMATION FOR AUTOANALYZERS

❖ **For Hitachi Model**

Calculation Method		Rate
Temperature		37°C
Volume (µL)	Specimen	8.0
	R1	160
	R2	40
Wavelength (nm)	Main	340
	Sub	405
Measurement (cycle)	Point 1	10
	Point 2	27
	Point 3	34
Calibration type		Linear
Unit		U/L

14. OTHER INSTRUCTIONS AND CAUTION

- Results may differ depending on the sample/reagent ratio. Adjust parameters for different analyzer.

- Perform the QC procedure on the day of determination.

15. PACKING AND KIT CONFIGURATION

Code	Package	Test/Kit*	Test/Kit**
11C015A	1x60mL; 1x15mL	280	540
11C015A2	2x60mL; 2x15mL	560	1080
11C015A3	3x60mL; 3x15mL	840	1620
11C015A4	4x60mL; 4x15mL	1120	2160
11C005A	5x60mL; 5x15mL	1400	2700
11C015A6	6x60mL; 6x15mL	1680	3240
11C015	1x80mL; 1x20mL	380	720
11C015-2	2x80mL; 2x20mL	760	1440
11C005	3x80mL; 3x20mL	1140	2160
11C015-4	4x80mL; 4x20mL	1520	2880
11C015-5	5x80mL; 5x20mL	1900	3600

* For middle-scale automatic analyzers such as: SK300; BS series; BA200; BA400. Chemwell Series; Dirui Series; Biolyzer series, HumanStar 300, Erba Series; Bioelab Series, BX 3010; Pictus P500;…

** For large-scale automatic analyzers such as: CA800; CA400; Randox Imola; Randox Modena+; BM 6010; Biolis50i; SK500; AU Series; Pictus P700; C series; Ci series; HumanStar 600; Kenolab series ...

The above-mentioned test's number are calculated base on technical specifications of each analyzer. The real number of test per kit may higher than the calculation's number.

The above-mentioned test's number cover the loss of the dead volume of reagent bottles but not cover the loss of Calibrator and Control.

Please feel free to contact authorized distributor for further confirmation.

16. REFERENCES

1. Y. Xue, ... Navaid Iqbal, in Reference Module in Biomedical Sciences, 2014
2. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices, EP05-A2, 2004
3. CLSI EP17 · Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition, 2017
4. In house data, UMA Diagnostics

17. MANUFACTURER

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