UMA CO., LTD.

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Matsudo, Chiba, Japan

MEASURE CK

Reagent for determination of Creatine Kinase

IFCC Method

2 - 8°C	**IVD** *In vitro* Diagnostics	**QUALITY MANAGEMENT SYSTEM (BY TUV)**
DO NOT freeze	12 months/block from light	**ISO 13485:2016**

1. PURPOSE OF USE

Providing a quantitative in vitro assay for the Creatine Kinase (CK) 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

CK catalyzes the synthesis of ATP and PCr in a reversible Lohmann reaction. The findings revealed that the fast-muscle EDL had the maximal CK activity, followed by diaphragm, with the lowest in the SOL. Electrophoretic separation of CK isoenzymes in all three muscles revealed the existence of only CK-MM isoenzyme. Further separation of CK-MM isoenzyme for subforms showed only CK-MM3 in all three muscles. Compared to muscles, serum had very little CK activity; however, the CK consisted of three distinct isoenzymes: CK-BB (15.3%), CK-MB (3.9%), and CK-MM (80.8%). Further electrophoresis of the serum CK-MM isoenzyme revealed the presence of three subforms: CK-MM1 (6.3%), CK-MM2 (24%), and CK-MM3 (69.7%) (Gupta et al., 1994). Literature abounds showing that the CK-MM3 subform secretes from muscles into the plasma, where it converts into the MM2 and MM1 subforms by carboxypeptidase-N2.

Within 1 h of exposure to carbofuran (1.5 mg/kg, s.c.), CK activity was significantly reduced in the SOL, while it increased in the diaphragm. At the same time, activities of CK and all three CK isoenzymes were significantly elevated in serum. An important finding was that carbofuran or methyl parathion caused a shift in the serum CK-MM subform; i.e., higher sequential conversions of CK-MM3 subform to CK-MM2 and CK-MM2 to CK-MM1, possibly due to enhanced carboxypeptidase-N2 activity.

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: Imidazsole; adenosine-di-phosphate(ADP) D-Glucose, Hexokinase (HK); nicotinamide adenine dinucleotide phosphate (NADP) Glucose-6-phosphate dehydrogenase (G6PDH)

  Reagent R-1 is ready for use

- Reagent R-2: Phosphocreatine

  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 specimen.

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

- Analyze samples soon after collection.

- Stability:

- 8 hours at 15 - 25°C
- 3 days at 2 - 8°C
- 6 months at < -20°C

- See interferences section for details about possible sample interferences.

6. MEASUREMENT PRINCIPLE

CK in serum produces Creatine and ATP by Phosphocreatine and ADP as substrates. Then, ATP is transformed into ADP and Glucose-6-phosphate by the action of Hexokinase (HK) existing under Glucose.

This Glucose-6-phosphate is oxidized into 6-phosphogluconate by Glucose-6-phosphate dehydrogenase (G6PDH). At this point, NADP is reduced to NADPH simultaneously and CK activity can be obtained by measuring increase rate of absorbance of NADPH.

1st reaction

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

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

2nd reaction

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

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	$\xrightarrow[280s]{37°C}$	Reagent 2
4.0µL		160µL		40µL

$\xrightarrow[180s]{37°C}$ Meas. Abs I $\xrightarrow[140s]{37°C}$ Meas. Abs II

(340/405nm) (340/405nm)

$\longrightarrow$ ΔAbs at 340/405nm $\longrightarrow$ Activity of CK

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 = f(ΔAbs)

- Calculate CK 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 enzyme activity range of 10 - 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)	=	1.0 U/L
Limit of Detection (LoD)	=	3.5 U/L
Limit of Quantitation (LoQ)	=	10 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: Change in absorbance when using purified water is 0.001 - 0.003 Abs/minute, and change in absorbance using sample of 500 U/L is higher than 0.047 Abs/minute.

- 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 3% and Total Precision is less than 5%.

Repeatability	*Mean* U/L	*SD* U/L	*CV* %
Control Lyo L-1	68.9	1.25	1.81
Control Lyo L-2	264.2	2.62	0.99

Reproducibility	*Mean* U/L	*SD* U/L	*CV* %
Control Lyo L-1	68.9	2.91	4.22
Control Lyo L-2	264.2	7.39	2.80

Total precision	*Mean* U/L	*SD* U/L	*CV* %
Control Lyo L-1	68.9	3.04	4.41
Control Lyo L-2	264.2	7.61	2.88

e. Correlation Test

Serum

Regression equation: y = 0.9989x + 1.0499 (n = 52)

Correlation coefficient: r = 0.9987

Plasma

Regression equation: y = 1.0006x + 0.6420

Correlation coefficient: r = 1.000

(y: value from this method)

Reference Material for Calibration

ReCCS JCCLS CRM-001

10. EXPECTED VALUES

- Male: 59 - 248 U/L

- Female: 41 - 153 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 up to 500 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	4.0
	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.

- Prepare the calibration curve on the day of determination.

15. PACKING AND KIT CONFIGURATION

Code	Package	Test/Kit*	Test/Kit**
11C031A	1x60mL; 1x15mL	280	540
11C031A2	2x60mL; 2x15mL	560	1080
11C031A3	3x60mL; 3x15mL	840	1620
11C031A4	4x60mL; 4x15mL	1120	2160
11C011A	5x60mL; 5x15mL	1400	2700
11C031A6	6x60mL; 6x15mL	1680	3240
11C031	1x80mL; 1x20mL	380	720
11C031-2	2x80mL; 2x20mL	760	1440
11C011	3x80mL; 3x20mL	1140	2160
11C031-4	4x80mL; 4x20mL	1520	2880
11C031-5	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. Ramesh C. Gupta, ... Jitendra K. Malik, in Handbook of Toxicology of Chemical Warfare Agents (Second Edition), 2015
1. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices, EP05-A2, 2004
2. CLSI EP17 · Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition, 2017
3. In house data, UMA Diagnostics

17. MANUFACTURER

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