# Creatine Kinase-MB Assay Kit (CK-MB)

**Method:** DGKC

| Cat .No. | Size | Instrument |
|---|---|---|
| GB210X | R1: 2×40 ml<br>R2: 1×20 ml | For Hitachi 717<br>& ShimadzuCL7200/8000 |
| GS211X | R1: 4×20 ml<br>R2: 1×20 ml | For Hitachi917<br>& OlympusAU640/400/600 |
| GH211X | R1: 2×40 ml<br>R2: 1×20 ml | For Hitachi902 |
| GX211X | R1: 1×80 ml<br>R2: 1×20 ml | For SYNCHRON CX4-5-7-9/LX20/DXC600-800 |
| GT211X | R1: 2×40 ml<br>R2: 1×20 ml | For TOSHIBA |

## INTENDED USE

For the *in vitro* quantitative determination of creatine kinase -MB（CK-MB）in human serum .

## CLINICAL SIGNIFICANCE

CK-MB is present in low concentration in normal human serum but is increased as a result of heart injury, and rarely, skeletal muscle damage. CK-MB is widely used as an indicator of acute myocardial infarction as the detection of elevated activities is considered highly specific for this condition.

## ASSAY PRINCIPLE

Creatine Kinase is a dimer. Its monomeric subunits are designated M and B. The subunits combine to form three isoenzymes namely CKBB, CK-MB and CK-MM. CK-MM and CK-MB are found primarily in skeletal and heart muscle, respectively, while CK-BB is found mainly in the brain and smooth muscle tissue. In serum, the Ck-BB activity can be ignored. M Subunits of CK-MM and CK-MB are inactivated by reaction with anti-M antibody (immunoinhibition). The remaining B-subunit is measured enzymatically.

$$\text{creatine phosphate} + \text{ADP} \xrightarrow{\text{Creatine kinase}} \text{creatine} + \text{ATP}$$

$$\text{ATP} + \text{glucose} \xrightarrow{\text{Hexokinase}} \text{ADP} + \text{glucose-6-phosphate}$$

$$\text{glucose-6-phosphate} + \text{NAD} \xrightarrow{\text{G-6-PD}} \text{6-phosphogluconate} + \text{NADH}$$

Through a series of coupled enzymatic reactions, NADH is produced at a rate directly proportional to the CK B subunit activity. Multiply the activity by 2, and this result is just the activity of CK-MB.

## SAMPLE COLLECTION AND PREPARATION

Serum samples. Samples are stable for 4 hours at temperature, for 8 hours at 2-8°C, for 2 days at -20°C.

## REAGENT COMPOSITION

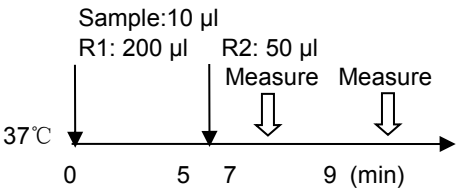
| Contents | Concentration |
|---|---|
| Imidazole buffer pH6.7 | 100 mmol/L |
| Creatine phosphate | 30 mmol/L |
| Glucose | 20 mmol/L |
| N-Acetylcysteine (NAC) | 20 mmol/L |
| MgAC | 10 mmol/L |
| EDTA | 2 mmol/L |
| ADP | 2 mmol/L |
| AMP | 5 mmol/L |
| Diadenosine pentaphosphate | 10 μmol/L |
| G6PDH | ≥1.5 KU/L |
| HK | ≥2.5 KU/L |
| NADP | 2 mmol/L |
| Anti-M subunit polyclonal antibody | ≥ 2KU/L |

## STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.
Stable up to the expiry date when stored at 2-8℃.
The reagent after opening is stable for 28 days on-board the analyser .

## ASSAY PROCEDURE

**Test Procedure for Analyzers** (HITACHI 917)
Assay Mode: Rate
Wave Length (main/sub): 340 nm/ 410 nm




1. Mix 10 μl sample with 200 μl R1 and incubate at 37℃ for 5 minutes.
2. Add 50 μl R2 into cuvette, mix and incubate at 37℃ for 2 minutes.
3. Read initial absorbance and start timer simultaneously, read again after 1, 2 and 3 minutes.
4. Calculate absorbance change per minute (ΔA/min)

## CALCULATION

**Using K factor**

$$\text{CK-MB (U/L)} = \frac{\Delta A/\text{min} \times V_t}{\varepsilon \times V_s \times L} \times 1000 \times 2$$

$$= \Delta A/\text{min} \times K$$

K = 8255 (37℃)

## QUALITY CONTROL

For quality control, use Control as daily quality control sera and can be purchased separately. Values should fall within a specific range. If these values fall outside the range and repeti-tion excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.
4. Check the quality of the water used for reagents reconstitution.

## NORMAL VALUE

CK-MB: < 25 U/L
It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

Beijing Strong Biotechnologies, Inc.
Add: 5/F Kuang Yi Building, No. 15 Hua Yuan Dong Lu, Haidian District, Beijing 100191 P. R. China
Tel: +86 10 8201 2486 Fax: +86 10 8201 2812
Web: www.bsbe.com.cn Email: jq.tech@bsbe.com.cn

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## SPECIFIC PERFORMANCE CHARACTERISTICS

### LINEARITY
The method is linear up to 600 U/L. If the sample above this concentration should be diluted 1+1 with 0.9% NaCl and repeat assay. Multiply the result by 2.

### PRECISION
The CV of the test should be less than 10%

| **Intra assay precision** | |
|---|---|
| N=15 | Level 1 |
| Mean(U/L) | 115.43 |
| SD(U/L) | 0.385 |
| CV | 0.33% |
| **Inter assay precision** | |
| N=5 | Level 1 |
| Mean (U/L) | 115.61 |
| SD(U/L) | 0.266 |
| CV | 0.23% |

### SENSITIVITY
The minimum detectable level that can be distinguished from zero has been determined as 5 U/L.

### INTERFERENCE
The following analytes were tested up to the levels indicated and found not to interfere:

| | |
|---|---|
| Intralipid: | 2000 mg/dl |
| Hemoglobin: | 500 mg/dl |
| Direct bilirubin: | 40 mg/dl |
| Ascorbic Acid: | 50 mg/dl |

### CORRELATION
This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:
Y=1.0048X+0.2362, and a correlation coefficient of 0.9989; 50 patient samples were analyzed.

### SAFETY PRECAUTIONS AND WARNINGS
1. For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.
2. The reagents contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.

## REFERENCES
1. Tietz, N. W, Textbook of Clinical Chemistry, W.B. Saunders Co,Philadelphia, 1986, p. 678-6863.
2. Szasz, G., et al. Clin. Chem 1976; 22: 650.
3. Recommendations of the German Society for Clinical Chemistry.Standardization of methods for the estimation of enzyme activities in biological fluids:Standard method for the determination of creatine kinase activity. J Clin Chem Clin Biochem 1977; 15:255-60.

## INDEX OF SYMBOLS

| | |
|---|---|
|  | Manufacture |
|  | Catalogue Number |
|  | Lot number |
|  | Date of manufacture |
|  | Use by(Expiration date) |
|  | For In-Vitro Diagnostic use only |
|  | Stored at 2-8℃ |
|  | Attention:See instruction for use |
|  | Authorized Representative in the European Company |

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