Creatine Kinase-MB Assay Kit (CK-MB)

Method: Immunoinhibition Method

Cat .No.	Package Size	Analyzer
EGS211X	R1: 4×20 ml R2: 1×20 ml	For Hitachi917 & Olympus AU640/400/600
EGB210X	R1: 2×40 ml R2: 1×20 ml	For Hitachi 717 & Shimadzu CL7200/8000
EGH211X	R1: 2×40 ml R2: 1×20 ml	For Hitachi902
EGD211X	R1: 24×4.3 ml R2: 6×4.3 ml	For Siemens Dupont/Siemens Behring Series
EGCKMB460	R1: 2×40 ml R2: 1×20 m	For Mindray BS120/180/190/200/220/230/240/430/460/830
EGGCKMB	R1: 2×40 ml R2: 1×20 ml	For Semi Auto Analyzer

INTENDED USE

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

CLINICAL SIGNIFICANCE

Detecting the activity of creatine kinase isoenzymes in serum or plasma is one of the most valuable enzymatic indicators for clinical diagnosis of acute myocardial infarction. Combined determination with creatine kinase is helpful for the differential diagnosis of myocardial infarction.

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.

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

1. Stable up to the expiry date when the reagent is sealed and stored in dark at 2-8 ℃.
2. The production date and expiry date are shown on the label.
3. Once opened, the reagents are stable for 28 days when refrigerated on the analyzer or refrigerator.
4. Reagents should not be contaminated.

APPLICABLE INSTRUMENT

This kit is theoretically suitable for all biochemistry analyzers and spectrophotometers covering the wavelength range of 340nm.

It is recommended to use this kit on a biochemistry analyzer for testing according to laboratory conditions.

SAMPLE COLLECTION AND PREPARATION

Serum or plasma (heparin anticoagulation).

Samples can be stored at 2°C-8°C for 8 hours and stored at (-15)-(-25)°C for 7 days.

ASSAY PROCEDURE

Test Condition (Hitachi 917)

Main wavelength	340 nm	Sample (S)	10 μl
Secondary wavelength	405 nm	Reagent 1（R1）	200μl
Reaction temperature	37℃	Reagent 2 (R2)	50μl
Cuvette diameter	1cm	Reaction type	Rate Method

Operate procedure

Add into cuvette：	
Sample (S)	10μl
Reagent 1 (R1)	200μl
Mix well and incubate for 5 minutes at 37℃	
Reagent 2 (R2)	50μl
Mix well and incubate for 2 minutes at 37℃, Continuously measure the absorbance within 180 seconds, and calculate the absorbance change rate (△A/min)	

Note: Parameters above are only introduced with Hitachi 917 as an example. The parameters of different biochemistry analyzers are slightly different. Please read the manual carefully before setting parameters.

CALIBRATION

1. Calibration mode: K factor calibration.
2. Requirements for calibration and frequency: It is recommended to calibrate at least once every two weeks. When the following situations occur, it is recommended to re-calibrate: change the reagent batch number, the indoor quality control runs out of control, the biochemistry analyzer carries out major maintenance or replaces the main parts such as light source or cuvette.

QUALITY CONTROL

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It is recommended to use Gcell CKMB control**.** The absorbance of quality control should be within the labeled value range. If the results deviate from the scope, please find out the reason by following steps:
1 Check the parameter setting and light source.
2 Check the cleanliness of the cuvette and sampling needle.
3 Check whether water is contaminated or not. Bacterial growth can lead to incorrect results.
4 Check the reaction temperature.
5 Check the validity of the kit

CALCULATION RESULT

CK-MB Active (U/L） = ΔA/min × K factor

Wavelength	340 nm
K Factor	8255(37℃)

REFERENCE RANGE

CK-MB: < 25 U/L

Laboratories are suggested to establish its own reference interval according to age, sex, diet and region.

INTERFERENCE

The effect of Intralipid ≤250 mg/dL, TBil ≤ 40 mg/dl, VitC≤30 mg/dl, is less than 10%. Any concentration of hemoglobin can interfere with the detection of CK-MB.

ACCURACY

Compared with competitors, in the range of [10,600.0] U/L, the correlation coefficient r≥0.975; In the range of [10,60] U/L, the absolute deviation should ≤ ±6 U/L; In the range of (60, 600] U/L, the relative deviation should ≤10%.

SENSITIVITY

When the sample concentration is 100 U/L, the change of absorbance should ≥ 0.010.

LINEARITY

In the range of [10,600.0] U/L, the linearity correlation coefficient r ≥ 0.99. In the range of [10,60]U/L, the absolute deviation should ≤ ±6U/L; In the range of [60,600] U/L, the relative deviation should ≤10%.

PRECISION

Repeatability precision was obtained by testing control or sample for 20 times of repeated measurement. Intermediate precision was obtained by testing human samples or control for 2 batches 5 days, and each batch was measured for 5 times.The results are as follows:

a) Repeatability precision (N=20)

Sample	Mean (g/L)	CV(%)
Control 1	148.335	0.93

b) Intermediate precision (N=25)

Sample	Mean(g/L)	CV(%)
Control 1	136.11	2.9
Serum	18.06	4.7

SAFETY PRECAUTIONS AND WARNINGS

1. The reagent contains preservatives. If it enters the eyes, mouth or contact on the skin, please rinse it thoroughly with clean water immediately and go to the hospital if necessary.
2. The reagent contains preservatives, which can react strongly with copper, lead and other metals to form azide metal. Therefore, please dilute the waste liquid and flush the drain pipe to avoid residual when disposal.
3. Do not mix or exchange reagents with different batches in the process of detection.
4. Opened reagents should be sealed and stored according to the specified method. Expired product should not be used.
5. Please dispose test tubes and other instruments that have touched the test sample according to the relevant medical waste disposal regulations. The following treatment methods can be selected:
Autoclave at 121°C for 15 minutes (but do not use autoclave to treat waste containing hypochlorous acid solution), or soak in hypochlorous acid solution (effective concentration greater than 1000ppm) for one hour the above.
6. Calibrator and control use human matrix serum, passed the detection of HIV (HIV 1, HIV 2) antibodies, hepatitis B surface antigen (HbsAg) and hepatitis C virus (HCV). All of them are negative. Although the detection method is highly accurate, it can not be guaranteed that all infected donors are found, so the control should also be treated as infectious specimens.

REFERENCES

1. Wurzburg, U, et al, Clin. Chem. 1976; 54: 357.

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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