Bioway Chemistry Reagent Series

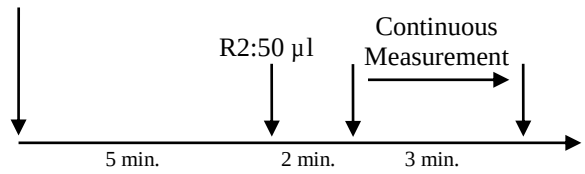
The Serum Creatine Kinase MB Reagent Kit

Detection of Creatine Kinase MB in Human Serum or Plasma on Chemistry Analyzers

Cat. No. R020K11 ***The Serum CK-MB Reagent Kit***

SUMMARY OF TEST PROCEDURE

Figure 1: Procedure Diagram



*Refer to Figure 1 and the paCK-MBage insert for detail

Table 1: Instrument Parameters*

F factor	8360	Slope of reaction	Increase
Testing wavelength	Dλ : 340 nm Sλ : 405 nm	Sample volume	10 µl
Test method	Rate Method	R1 volume	200 µl
Reaction temperature	37℃	R2 volume	50 µl

INTENDED USE

Bioway Chemistry Reagent Series **CK-MB Reagent Kit** (the Kit) is an assay intended for *in vitro* quantitative detection of Creatine Kinase MB in human serum or plasma on automated clinical chemistry analyzers.

SUMMARY AND EXPLANATION

Creatine kinase is also known as creatine phosphokinase or phospho-creatine kinase because it catalyzes the reaction of creation and adenosine triphosphate to from phosphocreatine. It consists of two subunits which are B (brain type) and M (muscle type) and able to create three isoenzymes: CK-MM, CK-MB and CK-BB. Creatine kinase MB is found primarily in the skeletal muscle and heart muscle. Creatine kinase MB is widely measured in patients with chest pain for diagnosing myocardial infraction.

TEST PRINCIPLES

The Kit utilizes immunoinhibition method to measure the CK-MB activity (U/L) in human serum or plasma. CK-M is blocked by anti-CK-M antibody and measure the left CK-B activity by the following reaction:

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

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

$$\text{Glucose-6-phosphate + } NADP^+ \xrightarrow{G_6PDH} \text{6-phosphogluconate + NADPH + } H^+$$

Creatine kinase B catalyzed the phosphorylation of ADP to form ATP in the presence of creatine phosphate. Then a phosphate group is transferred from ATP to glucose and produces glucose-6-phosphate which is oxidized to 6-phosphogluconate accompanying with the production of NADPH. The process is quantified by measuring the absorabances at 340 nm in a kinetic fashion.

The rate of increase in absorbance at 340 nm is directly proportional to the amount of CK-B in the sample. Then multiply the result by 2 to calculate the real activity of CK-MB.

MATERIALS PROVIDED

Reagents:

R1	Creatine phosphate NADP Glucoe-6-phosphate dehydrogenase Sodium azide	100 mmol/L 2 mmol/L 2000 U/L 2 mmol/L
R2	Magnesium acetate CK-M antibody	100 mmol/L 5 mmol/L

MATERIALS NEEDED BUT NOT PROVIDED

1. Automated chemistry analyzer
2. CK-MB control and calibrator set (commercially available)

INSTRUMENT

The Kit is applicable on most automated chemistry analyzers. Refer to specific instrument application for suggested settings.

STORAGE AND STABILITY

Store the reagents at 2-8℃. Avoid direct sunlight. The Kit is stable through the expiration date when stored properly. The reagent is stable for 1 month at 2-8℃ after opening.

PRECAUTIONS

1. The Kit is for *in vitro* diagnostic use only. Not for use in humans or animals.
2. The instructions must be followed to obtain accurate results.
3. Do not use the reagents beyond the expiration date.
4. Treat all specimens as infectious. Proper handling and disposal procedures of specimens and test materials should be strictly followed.
5. Reagents contain sodium azide as preservative; avoid contact with skin and eyes, flush with copious amounts of water when disposing.

SPECIMEN COLLECTION AND HANDLING

Follow standard laboratory procedures to collect serum preventing hemolysis.

It is recommended to perform test immediately after sample collection. If the test cannot be done immediately, specimens may be stored at 2~8℃ for 10 days.

TEST PROCEDURE (see Figure 1)

Reagent 1 and 2 are liquid stable ready-to-use, no preparation needed.

Calibration: Recommend using commercially available calibrator set for optimal results.

Test procedure: see Figure 1 and Table 1 for instrument parameter setup. Refer to specific instrument application for suggested setting.

1. Add 10 µl of sample and 200 µl of R1; mix well and incubate at 37℃ for 5 minutes.
2. Add 50µl of R2; mix well and incubate at 37℃ for 2 minutes.
3. Take continuous optical density measurement for 3 minutes.
4. Calculate average Δ A /min

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RESULT

The CK-MB activity in U/L can be obtained by the following calculation:

CK-MB (U/L） = (ΔA_{test} / min - ΔA_{blank} / min)× factor (F)

The calculation factor for UV spectrophotometer is 8360 when the optical path is 10 mm. It is recommended for each laboratory to establish its own F factor. Please refer to instrument application if testing under different conditions.

EXPECTED VALUES

<25 U/L
It is recommended for each laboratory to establish its own expected values.

QUALITY CONTROL

Using commercially available controls with known concentration is recommended before each batch of tests to ensure the test is properly performed and all reagents and the instrument are functional as specified.

LIMITATIONS

1. The Kit is for *in vitro* use on automated chemistry analyzers only.
2. The test result from the Kit should not be used as the only basis for definite diagnosis.
3. Samples with CK-MB exceeding the maximum measurement range should be diluted with saline and retested.

PERFORMANCE CHARACTERISTICS

Linearity: 0 - 500 U/L (R≥0.990)

Accuracy: Bias proportion 90%~110%

Precision: Within Run: CV≤6%;
Run-to-Run: CV≤8%

Interference: no interference detected for: Conjugated bilirubin (≤855umol/L), unconjugated bilirubin (≤342umol/L), ascorbic acid (≤50mg/dl), and lipid (≤2000NTU)

Sensitivity: At 340nm wavelength and 10mmoptical diameter, O.D. ≤0.40.

REFERENCES

1. Sato M., Rinsho Byori. 39(11):1129-34 (1991)
2. M. Panteghini *et al.*, Eur. J. Clin. Chem. Clin. Biochem. 32:383-389 (1994)
3. P. Rizzotti *et al.*, Eur. J. Clin. Chem. Clin. Biochem. 32:97-106 (1994)

Not Intended for Sale in the United States.

Pointe Biotech (Nanjing) Co., Ltd.

No.85,Xingmin South Road, Jiang Ning District, Nanjing,P.R.China 211100
Tel:86-25-52425019,
Fax:86-25-52424836
info@biowaydx.com