DATA SHEET

CK-MB ELISA

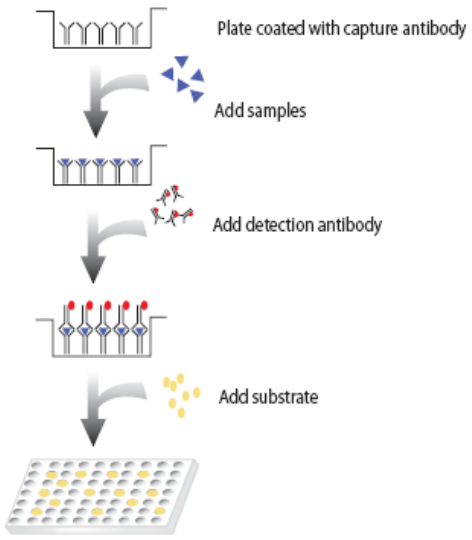
Catalog Number EA-0304 (For Research Use Only)

Introduction

During a heart attack (or myocardial infarction), damaged heart tissue releases cardiac enzymes such as creatine kinase (CK) (1-2). CK is found in the heart muscle, liver, skeletal muscles (such as in the arms and legs), and in the brain. CK-MB is a subtype of CK that is found only in heart muscle. Low level of the enzyme is normally found in the bloodstream, but if heart muscle is injured (as in a heart attack), the enzymes leak out of damaged heart muscle cells and their levels in the blood rise. To diagnose or confirm a recent heart attack is to test whether the level of the enzyme is increased. CK-MB, released after acute myocardial infarction (AMI), is detectable in blood as early as 3-4 hours after the onset of symptoms, and remains elevated for approximately 65 hours post infarct (3-4). CK-MB mass levels are reportedly 50% diagnostic for AMI after 3 hours and > 90% diagnostic at 6 hours (5). Such accuracy makes CK-MB mass determinations useful in confirming AMI in patients presenting to the ER with non-diagnostic ECGs > 6 hours after the onset of symptoms (6-7). Measurements of Creatine Kinase (CK) activity are primarily used for diagnosing skeletal muscle disease, myocardial infarction, cerebrovascular accidents, muscular dystrophy, hypothyroidism, pulmonary infarctions, as well as for monitoring the causes and treatments.

Principle of the assay

The CK-MB ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a mouse monoclonal antibody against distinct determinants on CK-MB for immobilization on the microtiter wells and a goat anti-cTnI antibody conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with these antibodies, resulting in CK-MB being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of CK-MB is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



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Materials provided with the kit

• Antibody-coated microtiter plate with 96 wells.
• Liquid CK-MB standards containing; 0, 7.5, 15, 50, 100, and 200 ng/ml CK-MB. 1.0 ml for each standard dose. Store at -20ºC or below.
• Enzyme Conjugate Reagent, 22 ml.
• TMB Reagent (One-Step), 11 ml.
• Stop Solution (1N HCl), 11 ml.

Material required but not provided

• Microplate reader capable of measuring absorbance at 450 nm
• Deionized or distilled water.

Warning and precautions

1. Caution: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. It is recommended that the reagents and patient samples be handled according to the OSHA Standard on Bloodborne Pathogens (8) or other appropriate national biohazard safety guidelines or regulations (9-11).
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.

Reagent preparation

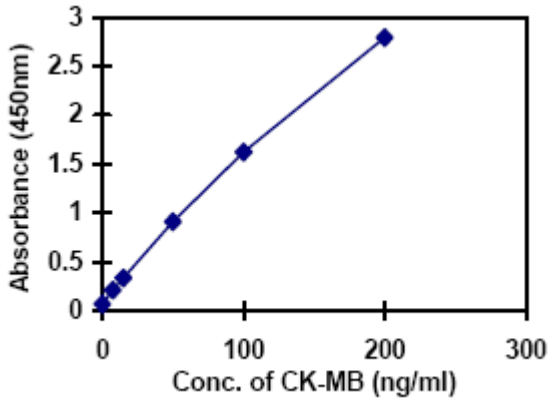
All reagents should be allowed to reach room temperature (18-25^{o}C) before use.
Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. The Reconstituted standards will be stable for up to 8 hours when stored sealed at 2-8 ^{o}C.
Discard the reconstituted Standards after 8 hours. To assure maximum stability of the reconstituted Standards, they should be aliquoted and frozen (−20 ^{o}C or below) immediately after reconstitution has been achieved. Each aliquoted Standard should be frozen and thawed only once.

Assay procedure

1. Dispense 20 µl of standard, specimens, and controls into appropriate wells.
2. Dispense 200 µl of Enzyme Conjugate Reagent to each well.
3. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
4. Incubate at room temperature (18-25^{o}C) for 60 minutes.
5. Remove the incubation mixture by emptying plate content into a waste container.
6. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
9. Incubate at room temperature for 20 minutes.
10. Stop the reaction by adding 100 µl of Stop Solution to each well.
11. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

Example of standard curve

CK-MB (ng/ml)	Absorbance (450 nm)
0	0.067
7.5	0.211
15	0.335
50	0.909
100	1.621
200	2.794



References

1. Lee, T.H., Goldman, L., Serum enzyme assays in the diagnosis of acute myocardial infarction. *Ann.Intern. Med*., 1986; 105: 221-233.
2. Meerson, F.Z., Javich, M.P., Isoenzyme pattern and activity of myocardial creatine phosphokinase under heart adaptation to prolonged overload. *BasicRes.Cardiol*., 1982; 77: 349-358.
3. Mair, J., Wagner, I., Jakob, G., et al., Different time courses of cardic contractile proteins after acute myocardial infarction. *Clin. Chim. Acta.*, 1994; 231: 47-60.
4. Levitt, M.A., Promes, S.B., Bullock, S., et al., Combined cardiac marker approach with adjunct two-dimensional echocardiography to diagnose acute myocardial infarction in the emergency department. *Ann, Em. Med*., 1996; 27: 1-7.
5. Li, D., Jialal, I., Keffer J.H., Greater frequency of increased cardiac Troponin T than increased cardiac Troponin I in patients with chronid renal failure. *Clin. Chem*., 1996; 42:114-115.
6. Mair, J., Smidt, J., Lechleitner, P., et al., A decision tree for the early diagnosis of acute myocardial infarction in nontraumatic chest pain patient at hospital admission. *Chest*, 1995; 108:1502-1509.
7. Yang, Z., Zhang, W., Liu Y., Prognostic efficacy of Troponin T measurement in angina pectoris. *Chin. Med. J. (Engl)*, 1995; 108: 626-630.
8. U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030. Occupational Exposure ot Bloodborne Pathogens; Final Rule. Federal Register; 56(235):64175, 1991.
9. USA Center for Disease Control/National Institute of Health Manual "Biosafety Microbiological and Biomedical Laboratories", (1984).

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