Intended Use: The Quantitative Determination of Circulating Creatinine Kinase (MB-isoform) Concentrations in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

Test System

Creatinine Kinase MB (CK-MB) Test System
Product Code: 2925-300

1.0 INTRODUCTION

Creatinine kinase (CK) is an enzyme, found primarily in muscle and brain tissue, which exists as three dimeric isoenzymes — CK-MM (CK-3), CK-MB (CK-2), and CK-BB (CK-1) — built from the three catalytic subunits M (muscle), B (brain), and M (muscle). CK-MB is a dimer composed of two subunits designated M and B. CK-MM, the isoenzyme which contains a muscle-specific molecule, is used in medicine to assess the presence of myocardial infarction, or a heart attack.

1.1 MEASUREMENT OF KM FOR CK-MM

In AMI, plasma CK-MB typically rises some 3 to 8 hours after the onset of chest pains, peaks within 9 to 30 hours, and returns to normal levels within 72 hours. The pattern of serial CK-MB determinations is more informative than a single determination. One CK-MB measurement, even when taken at an appropriate time, cannot be considered confirm or rule out the occurrence of AMI. High levels might reflect skeletal muscle injury rather than myocardial damage. A value within the reference range might be significant if it represents an increase from the patient's baseline levels. According to current standards, creatinine kinase isoenzymes and related peptides of CK-MB are added to the reactants mixed. Reaction between the various CK-MB antibodies and native CK-MB forms a sandwich complex that binds with the streptavidin coated to the well.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Immunoenzymometric assay (TYPE 3):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and unconjugated) and different antigen coating conjugates recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CK-MB antibody. Upon mixing biotin labeled monoclonal antibody, the enzyme-labeled antibody binds to the sandwich complex bound to the solid surface and is separated from the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[ E_{\text{Ab}(m)} \times \text{Ag}(\text{MB}) \times \text{BtnAb}(m) = \text{Immobilized complex} \]

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After the completion of the required incubation period, the enzyme-CK-MB immunoassay is separated from the unbound enzyme-CK-MB conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by a suitably produced color. The employment of several serum references of known (CK-MB) levels permits the constitution of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with CK-MB concentration.

3.0 PRINCIPLE

Serial measurement of biochemical markers is now accepted universally as an important determinant in ruling in or ruling out acute myocardial infarction. CK-MB is one of the most important myocardial markers (in spite of not being altogether cardiac-specific), with well established roles in confirming acute myocardial infarction and in monitoring separation during thrombolytic therapy following AMI.

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After equilibrium is attained, the antibody-bound fraction is

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In order for the assay results to be considered valid the QC PARAMETERS should not be used in lieu of a dose response curve corrected value.

1. The absorbance (OD) of calibrator 'A' should be < 0.075
2. If more than one (1) plate is used, it is recommended to repeat the same sequence to eliminate any time-deviation during reaction.
3. Highly lipemic, hemolyzed or grossly contaminated samples may be diluted with the zero calibrator and re-assayed.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted by the user, this data must be discarded.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor recovery of the analyte.
8. Use components from the same lot. No intermixing of reagents or solutions between different batches.
9. Patient samples with CK-MB concentrations above 400 ng/ml may be diluted with the zero calibrator and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may result in inaccurate results.
11. All applicable national standards, regulations, and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. The antibody system used did not detect any CK-MM or CK-BB isoforms. Only slight amounts of bias between the CK-MB AccuBind® ELISA test system and the reference method were indicated by the least square regression equation and correlation coefficient.
13. Accuracy
The CK-MB AccuBind® ELISA test system was compared with a radioluminomassay assay. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from ND – 86 ng/ml). The total number of such specimens was 124. The data obtained is Table 4.

14.4 Specificity
The cross-reactivity of the CK-MB AccuBind® ELISA method to selected substances was evaluated by adding the interfering substance(s) to a series of the following concentrations. The antibody system used did not detect any CK-BB or CK-MM isoforms at high levels.

15.0 REFERENCES