After the completion of the required incubation period, the enzyme-CK-MB antibody bound conjugate is separated from the unbound enzyme conjugated monoclonal antibody. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known (CK-MB) levels permits the construction of a dose response curve of activity and concentration. Comparison to the dose response curve, an unknown specimen’s activity can be correlated with CK-MB concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope 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 of biotin labeled monoclonal antibody, the enzyme-labeled antibody and a serum containing the native antigen reaction results between 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:

\[ \text{EnzAb (m) + AgCM-MB + BtnAb (m) \xrightarrow{k_a} \text{Immobilized complex} \]

\[ \text{Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:} \]

\[ \text{EnzAb (m) + AgCM-MB + BtnAb (m) \xrightarrow{k_a} \text{Immobilized complex} \]

\[ \text{Streptavidin immobilized on well} \]

\[ \text{Immunized complex = sandwich complex bound to the solid surface} \]

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. CK-MB Calibrators – 1.0 ml/vial (Lyophilized) (A – F)

B. CK-MB Enzyme Reagent – 15 ml vial (µC)

The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain red top tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive pipetting. When assayed in duplicate, 0.050 ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variation.

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable container. Store at 2-10°C for up to 60 days.

2. Working Substrate Solution

Pour the contents of amber vial labeled Solution ‘A’ into the clear vial labeled Solution ‘B’. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 25°C).

**Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microwells for calibrator, control and patient specimen to be tested. Use a 96-well disposable microwell plate for the assay. Use a microwell plate that has been tested to ensure reproducibility and sensitivity.

2. Pipette 0.025 ml (25µl) of the appropriate calibrators, controls and samples into the assigned wells.

3. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

4. Swirl the microwell gently for 20-30 seconds to mix. Cover with a plastic wrap.

5. Incubate for 15 minutes at room temperature (20-25°C).

6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times. Remove the last wash buffer. The plate washer can be used. Follow the manufacturer’s instruction for proper usage. If the wash is not removed, the plate will not be washed properly.

8. Add 50µl of Stop solution (see Reagent Preparation Section). DO NOT USE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Read the absorbance in each well at 450-650nm (a reference to 690-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

**NOTE:** Always add reagents in the same order to minimize reaction time differences between wells.
11.0 QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator ‘A’ should be ≤ 0.1.
2. The absorbance (OD) of calibrators ‘B’ and ‘C’ should be ≥ 1.3.
3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimens should not be used.
4. If more than one plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
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 replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from 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 yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory practice, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com

13.0 EXPECTED VALUES

CK-MB values are consistently higher in plasma than in serum; thus, serum is preferred. Compared with fasting values in non-obese non-diabetic individuals, CK-MB levels are higher in obese non-diabetic subjects and lower in trained athletes.

Each laboratory is advised to establish its own ranges for normal and abnormal populations. These ranges are always dependent upon locale, population, laboratory, technique and specificity of the method.

Based on the clinical data gathered by Monobind in concordance with the published literature, the following ranges have been assigned. These ranges should be used as guidelines only:

| Adult (Normal) | 2.0 – 5.2 ng/ml |

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the CK-MB AccuBind™ Immunoassay is determined by analysis on three different levels of pool control sera. The number (N), mean value, standard deviation (σ) and coefficient of variation (C.V.) for each of these control pools are presented in Table 2 and Table 3.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the 0 ng/ml serum calibrator and using the 0.19 ng/ml (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.182 ng/ml.

14.3 Accuracy

The CK-MB AccuBind™ ELISA test system was compared with a predicate radioimmunoassay assay. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from 1 – 86 ng/ml). The total number of such specimens was 124. The data obtained is displayed in Table 4.

15.0 REFERENCES

5. Gibber WB, Lewis LM, Eri RE, et al, “Early detection of acute myocardial infarction in patients presenting with chest pain and non-diagnostic ECGs; serial CK-MB sampling in concordance with published the following ranges have been assigned. These ranges should be used as guidelines only: Medscape, 19, 1359-66 (1990).