The employment of several serum references of known (CK-MB) levels permits the construction 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.

**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 excessively added biotinylated monoclonal anti-CK-MB antibody.

Upon mixing biotin labeled monoclonal antibody, the enzyme-labeled antibody and a serum containing the native antigen reaction results between the native antigen and the antibodies, with competition for or steric hindrance, to form a soluble sandwich complex.

The interaction is illustrated by the following equation:

\[ E_{Ab}^{(2)} + Ag_{Ab}^{(2)} + BnAb^{(2)} \rightarrow E_{Ab}^{(2)} + Ag_{Ab}^{(2)} + E_{Ab}^{(2)} + BnAb^{(2)} \]

**SPECIMEN COLLECTION AND PREPARATION**

The specimens shall be blood serum in type and the usual precautions in the collection of venous blood samples should be observed. The blood should be collected in a plain red vacuum tube with anticoagulants or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the cells from the serum.

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 repetitive freezing and thawing. When assayed in duplicate, 0.050 ml of the specimen is required.

**REAGENTS AND MATERIALS PROVIDED:**

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

Six (6) vials of references for CK-MB antigen manufactured at levels of (A), (B), (C), (100X), (200X), and (400X) ng/ml.

Reconstitute each vial with 1.0 ml of distilled or deionized water.

The reconstituted calibrators are stable for 7 days at -2°C.

In store for longer stability to time, aliquot the reconstituted calibrators in cryo vials and store at -10°C. DO NOT FREEZE THEM MORE THAN ONCE. A preservative is not added.

Note: The calibrators, human serum based, were calibrated using gravimetric protein weight from a >95% purified preparation as measured with PAGE.

B. CK-MB Tracer Reagent – 13 ml/vial Icon

One (1) vial containing enzyme labeled affinity purified antibody and biotin labeled monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Light Reaction Wells – 96 wells – Icon

One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml Icon

Saline. A preservative has been added. Store at 2-30°C.

E. Signal Reagent A – 7.0ml/vial – Icon S

One (1) vial that contains tetrathymabenzidine (TMB) solubilized in buffer. Store at 2-8°C.

F. Signal Reagent B – 7.0ml/vial – Icon S

One (1) amber vial that includes hydrogen peroxide (H₂O₂) dissolved in buffer. Store at 2-8°C.

**PRODUCT INSET:** (Instruction Booklet).

**REQUIRED BUT NOT PROVIDED:**

1. Pipettes with tips for delivering 25µl and 100µl volumes with a precision of better than 1.5%.
   2. Dispensers (for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5% (optional).
   3. Microplate washer or a squeeze bottle (optional).
   4. Microplate Luminometer
   5. Container(s) for mixing reagents (see below).
   6. Absorbent Paper for blotting the microplate wells.
   7. Plastic wrap or microplate cover for incubation steps.
   8. Vacuum aspiration
   10. Storage container for storage of wash buffer.
   11. Distilled or deionized water.

**REAGENT PREPARATION**

1. Wash Buffer

Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at room temperature 20-27°C.

2. Working Signal Reagent Solution - Store at 2 - 8°C.

Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per procedure performance. The working solution is made up by mixing 1 ml of Signal Reagent A into Signal Reagent B in a 1:1 ratio.

**TEST PROCEDURE**

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

1. Format the microplates’ wells for calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microplate strips back into the aluminum bag, seal and store at 2-8°C.

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

**Incubate for up to 30 days. Avoid repetitive freezing and thawing.** When assayed in duplicate, 0.050 ml of the specimen is required.

**Testing Procedure:**

A. Format the microplates’ wells for calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microplate strips back into the aluminum bag, seal and store at 2-8°C.

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

C. Incubate 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 repetitive freezing and thawing. When assayed in duplicate, 0.050 ml of the specimen is required.

D. Plot the light intensity for each duplicate serum reference versus the corresponding CK-MB concentration in ng/ml on linear graph paper.

**Note:** The calibrators, human serum based, were calibrated using gravimetric protein weight from a >95% purified preparation as measured with PAGE.

E. Draw the best-fit curve through the plotted points. To determine the concentration of CK-MB for an unknown, locate the average RLU’s (22664) of control 2 intersects the calibration curve at (76.5ng/ml) CK-MB concentration (See Figure 1).

**RESULTS**

A dose response curve is used to ascertain the concentration of CK-MB in unknown specimens.

1. Record the RLUs obtained from the microplate luminometer. The results should be read within thirty (30) minutes of adding the working signal reagent.

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

Each laboratory should assay controls at levels in the low, normal and elevated ranges for monitoring assay performance. These controls should be treated as unknowns and values determined in every procedure performed. Quality control trends should be maintained to monitor batch to batch consistency.

**Process**

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health. "Biohazards in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, NIH.
Q.C. PARAMETERS
In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established range.

LIMITATIONS OF PROCEDURE
1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of reagents should be done using a multichannel pipette to avoid assay drift.
3. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
4. Highly lipemic, hemolysed or grossly contaminated specimen(s) should not be used.
5. 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 result.
6. Use components from the same lot. No intermixing of reagents from different batches.

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.

Table 1

<table>
<thead>
<tr>
<th>CAL</th>
<th>SAMPLE</th>
<th>Mean RLU's</th>
<th>Low RLU's</th>
<th>High RLU's</th>
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A. Precision
The within and between assay precision of the CK-MB AccuLite™ CLIA procedure was determined by analyses on three different levels of control sera. The number results, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

B. Accuracy
The CK-MB AccuLite™ CLIA procedure was compared with a predicate ELISA immunoassay assay. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from N/D to 86 ng/ml). The total number of such specimens was 65. The data obtained is displayed in Table 4.

| Table 4

<table>
<thead>
<tr>
<th>Method Mean (x)</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
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<tr>
<td>This Method (y)</td>
<td>x = 10.63 y + 0.51</td>
<td>R = 0.951</td>
</tr>
<tr>
<td>Reference (x)</td>
<td>y = A + Bx</td>
<td>R = 0.951</td>
</tr>
</tbody>
</table>

*Only slight amounts of bias between the CK-MB AccuLite™ CLIA assay and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

REFERENCES