After the completion of the required incubation period, the immunoassay sandwich complex formed in step 3 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 reaction with a suitable substrate to produce light.

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 Creatine Kinase concentration.

### 3.0 PRINCIPLE

**Immunoenzymometric assay (TYPE 3)**

The essential reagents of an immunoassay or an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, and native antigen. In this procedure, the antibody 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 bioin labeled monoclonal antibody, the enzyme-labeled antibody and a serum containing the native antigen of interest, 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:

$$E_{\text{Ab}} + A + B_{\text{Ab}}(m) + \text{Enzyme conjugated anti-CK-MB} + \text{Antigen antibodies complex}$$

$$E_{\text{Ab}} + A + B_{\text{Ab}}(m) + \text{Enzyme conjugated anti-CK-MB} + \text{Antigen antibodies complex}$$

$$K_a = \text{Rate Constant of Association}$$

$$K_s = \text{Rate Constant of Dissociation}$$

### 2.0 SUMMARY AND EXPLANATION OF THE TEST

Creatinine kinase (CK) is an enzyme found primarily in muscle and brain tissue, which exists as three dimeric isoenzymes — CK-MM (Brain), CK-MB (Myocardium), and CK-BB (Skeletal muscle). CK-MB forms a sandwich complex that binds with the streptavidin labeled monoclonal antibody and forms a sandwich complex bound to the solid surface.

In AMI, plasma CK-MB typically raises some 3 to 8 hours after the onset of chest pain, with peak levels occurring within 6 to 30 hours. The pattern of serial CK-MB determinations is more informative than a single determination. 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 Creatine Kinase concentration.

### 4.0 REAGENTS

**Materials Provided:**

1. **Assay-Creatine-MB Calibrators — 1.0 mL (Lyophilized)**
2. **Assay-Creatine-MB Microplates — 96 wells**
3. **Assay-Creatine-MB Tracer Reagent — 13 mL/vial**
4. **Assay-Creatine-MB Standards — 100x**
5. **Assay-Creatine-MB Wash Buffer — 200x**
6. **Assay-Creatine-MB Signal Reagent A**
7. **Assay-Creatine-MB Signal Reagent B**
8. **Assay-Creatine-MB Control Reagent**
9. **Assay-Creatine-MB Negative Control**
10. **Assay-Creatine-MB Positive Control**

**Required But Not Provided:**

- Pipettes
- Pipette tips
- Microplate reader
- Pipette plate
- Calibration solutions
- Sterile disposable tips
- Ethanol
- Deionized water
- Microcentrifuge tubes
- Centrifuge
- Centrifuge tubes
- Test tubes
- Incubator
- Fridge
- Oven
- Water bath
- Test tubes
- Pipettes
- Pipette tips
- Pipette plate
- Centrifuge tubes
- Centrifuge
- Microplate reader
- Computer
- Fridge
- Oven
- Water bath
- Test tubes
- Pipettes
- Pipette tips
- Pipette plate
- Microplate reader
- Computer
- Fridge
- Oven
- Water bath
- Test tubes

### 5.0 PRECAUTIONS

**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 nonreactive 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 blood products are 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, "Biobreak in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS.

**Safe Disposal of kit components must be according to local regulatory and statutory requirement.**

### 6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and the usual precautions for collecting blood and urine samples should be observed. For acute cardiac conditions, the specimen can be collected in a heparinized 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 contaminates. Avoid repetitive freezing, thawing and re-freezing. Store in duplicate, 0.350mL (50µL) of the specimen is required.

### 7.0 QUALITY CONTROL

Each laboratory should assay controls at least in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be used for maintaining control of results. 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 variations.

### 8.0 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 2-30°C up to 60 days.

2. **Working Signal Reagent Solution - Store at 2 - 8°C**
   Determine the amount of reagent needed and prepare by mixing appropriate volumes of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 mL of A and 1 mL of B to make up eight well strips (a slight excess of solution is made). Discard the unused portion if not used within 36 hours after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

**Note:** Do not use reagents that are contaminated or have bacteriological growth.

### 9.0 TEST PROCEDURE

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

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

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

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

3. Add 0.100mL (100µL) of the tracer reagent to each well. It is very important to dispense and dispose of reagents close to the bottom of the microtubes.

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

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

6. After incubation, contents of the microtubes should be handled as potentially hazardous and capable of transmitting disease. For In Vitro Diagnostic Use, the specimen to separate the serum from the cells.

7. Add 0.350mL (350µL) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional washes with fresh wash buffer to the top by squeezing each well. Avoiding air bubbles. Decant the wash and repeat four (4) additional times.

8. Add 0.250mL (250µL) of signal reagent to all wells (see Reagent Preparation Section). Allow reagents to mix. Store reagents at room temperature (20-25°C).

9. Incubate the microtubes at room temperature (20-25°C) for thirty (30) minutes. 10. Read the relative light units in each well with a chemiluminescence microplate reader for 0.5-1.0 seconds. 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.

### 10.0 CALCULATION OF RESULTS

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

1. Record the RLUs obtained from the printout of the microplate reader run for the sample.

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

3. Draw the best-fit curve through the plotted points.

To determine the concentration of CK-MB for an unknown, locate the average RLUs of the unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (ng/mL) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

In the following example, the average RLUs (22664) of control

**1.0 INTRODUCTION**

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2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

10.3 EXPERTED VALUES

CK-MB values are consistently higher in plasma than in serum; non-diabetic subjects and lower in trained athletes.

10.4 PERFORMANCE CHARACTERISTICS

11.0 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 ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are 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.