Monobind Inc.
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AccuLite
CLIA Microwells

Creatine Kinase-MB (CK-MB) Test System

Product Code: 2975-300

1.0 INTRODUCTION

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

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 - CKMM (CK-3), CK-MB (CK-2), and CK-BB (CK-1) - built from subunits designated M and B. The CK-MB isoenzyme, which has a molecular mass of approximately 87,000 daltons, accounts for 5 to 50% of total CK activity in myocardium. In skeletal muscle, by contrast, it normally accounts for just 1% or less, CK-MM being the dominant form, though the percentage can be as high as 10% in conditions reflecting skeletal muscle injury and regeneration (e.g. severe exercise, muscular dystrophy, polymyositis).[2]

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 cardiac-specific), with well established roles in confirming acute myocardial infarction (AMI) and in monitoring reperfusion during thrombolytic therapy following AMI.[2]

In AMI, plasma CK-MB typically raises some 3 to 8 hours after the onset of chest pains, peaks within 9 to 30 hours, and returns to baseline levels within 48 to 72 hours.[7] 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 definitively confirm or rule out the occurrence of AMI. High levels might reflect skeletal 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. Accordingly, it has been recommended that CK-MB be measured on admission to the emergency room and at regulated intervals thereafter. The model described by the Heart Emergency Room (ER) Program[13] documented that serial testing for CK-MB isoenzyme mass on presentation and 3, 6 and 9 hours later in patients with symptoms suggestive of acute ischemic coronary syndrome presenting with a non-diagnostic or equivalent electrocardiogram was more effective (100% sensitivity with 100% negative predictive value) than continuous serial electrocardiograms, electrocardiography and graded exercise testing.

In this method, CK-MB calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies directed against distinct and different epitopes of CK-MB are added and 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.

After the completion of the required incubation period, the enzyme-CK-MB antibody bound conjugate 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 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 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:

$$^{Enz}Ab_{(m)} + Ag_{(CK\text{-}MB)} + {}^{Btn}Ab_{(m)} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(m)} - Ag_{(CK\text{-}MB)} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{CK\text{-}MB}$ = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(m)}$ = Enzyme labeled Monoclonal Atibody (Excess Quantity)
$^{Enz}Ab_{(m)} - Ag_{CK\text{-}MB} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies complex
k_a = Rate Constant of Association
k_d = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(m)} - Ag_{(CK\text{-}MB)} - {}^{Btn}Ab_{(m)} + \underline{Strept_{CW}} \Rightarrow$ <u>immobilized complex</u>
$\underline{Strept_{CW}}$ = Streptavidin immobilized on well
<u>Immobilized complex</u> = 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 antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. CK-MB Calibrators – 1.0 ml/vial (Lyophilized) Icons (A – F)
Six (6) vials of serum references for CK-MB antigen manufactured at levels of 0(**A**), 5(**B**), 25(**C**), 100(**D**), 200(**E**), and 400(**F**) ng/ml. Reconstitute each vial with 1.0ml of distilled or deionized water.
The reconstituted calibrators are stable for 7 days at 2-8°C. In order to store for a longer period of time, aliquot the reconstituted calibrators in cryo vials and store at -10°C. ***DO NOT FREEZE THAW MORE THAN ONCE.*** A preservative has been added.
Note: The calibrators, human serum based, were calibrated using gravimetric protein weight from a >99% 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/vial - Icon
One (1) vial containing a surfactant dissolved in buffered saline. A preservative has been added. Store at 2-8°C.

E. Signal Reagent A – 7ml/vial – Icon C[A]
One (1) vial containing Luminol solubilized in buffer. Store at 2-8°C.

F. Signal Reagent B – 7ml/vial – Icon C[B]
One (1) vial containing hydrogen peroxide (H_2O_2) dissolved in buffer. Store at 2-8°C.

G. Product Insert

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025ml (25µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Luminometer
5. Container(s) for mixing of reagents (see below).
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Storage container for storage of wash buffer.
11. Distilled or deionized water.
12. Quality Control Materials.

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 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, "Biosafety 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 in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

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 freezing and thawing. When assayed in duplicate, 0.050ml (50µl) 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. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. 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 for up to 60 days.
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 two (2) 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 bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-25°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' 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 all reagents close to the bottom of the microwell.**
Note: *Use a multichannel pipette to quickly dispense Enzyme Reagent to avoid drift if the dispensing is to take more than a few minutes.*
4. **Swirl the microplate 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 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat four (4) additional times.**
8. Add 0.100ml (100µl) of working signal reagent to all wells **(see Reagent Preparation Section)**. **Always add reagents in the same order to minimize reaction time differences between wells**
DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION
9. Incubate at room temperature in the dark for five (5) 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 luminometer as outlined in Example 1.
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.
4. 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 (in 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 2 intersects the calibration curve at (76.5ng/ml) **CK-MB** concentration (See Figure 1).

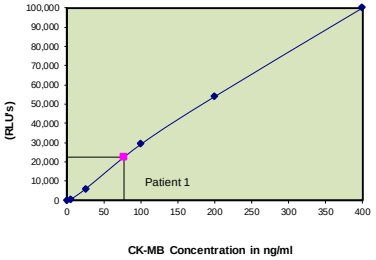
Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample ID	Well Number	RLUs	Mean RLUs	Value (ng/ml)
Cal A	A1	41	40	0
	B1	39		
Cal B	C1	611	617	5
	D1	623		
Cal C	E1	6170	6042	25
	F1	5913		
Cal D	G1	29242	29246	100
	H1	29250		
Cal E	A2	54479	54014	200
	B2	53550		
Cal F	C2	100468	100000	400
	D2	99532		
Ctrl 1	E2	311	324	2.94
	F2	336		
Ctrl 2	G2	22556	22664	76.5
	H2	22771		
Patient 1	A3	3244	3307	16.3
	B3	3371		

* The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLU's of the calibrators have been normalized to approximately 100,000 RLU's for the F calibrator (greatest light output). This conversion eliminates differences cause by efficiency of the various instruments that can be used to measure light output. The conversion varies between instruments and should be established for each instrument before using it as a factor.

Figure 1



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.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. 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.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. 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.
11. 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.
12. 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.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or a trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for AccuLite® CLIA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato, LM, Stuart, MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin. Chem.* 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. 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, Monobind shall have no liability.
6. 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.

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:**

TABLE 1
Expected Value Range for CK-MB AccuLite® CLIA Test System

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 AccuLite® CLIA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

SAMPLE	N	X	σ	CV
Low	12	0.78	0.08	10.3%
Medium	12	12.80	1.05	8.2%
High	12	49.80	2.56	5.1%

TABLE 3
Between Assay Precision (Values in ng/ml)

SAMPLE	N	X	σ	CV
Low	10	0.81	0.09	11.1%
Medium	10	12.10	1.17	9.7%
High	10	52.30	2.98	5.7%

*As measured in ten experiments in duplicate over ten days.

14.2 Sensitivity

The sensitivity (detection limit) of CK-MB AccuLite® CLIA test system was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2**σ** (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.5 ng/ml.

14.3 Accuracy

The CK-MB AccuLite® CLIA test system was compared against a reference method. Biological specimens from population (symptomatic and asymptomatic) were used (the values ranged from N/D – 86 ng/ml). The total number of such specimens was 65. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	10.63	y = -0.834 + 0.983(x)	0.951
Reference (x)	11.56		

Only slight amounts of bias between the CK-MB AccuLite® CLIA test system 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.

14.4 Specificity

The cross reactivity of the CK-MB AccuLite® CLIA test system to selected substances was evaluated by adding the interfering substance(s) to a serum matrix at high concentration(s). The antibody system used did not detect any CK-BB or CK-MM isoforms when tested at very high concentrations.

15.0 REFERENCES

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Revision: 4 **Date: 2019-Jul-16** **DCO: 1353**
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Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2°-8° Temperature Limitation Storage Condition (2-8° C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE European Conformity	