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CE

CK-MB
Chemiluminescence Immunoassay

Cat. No. 9024-C

Intended Use: The Quantitative Determination of Circulating Creatinine Kinase (MB-Isoform) Concentrations in Human Serum by a Microplate Immunoenzymometric assay

SUMMARY AND EXPLANATION OF THE TEST

Creatine 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 CK-MB is one of the most important myocardial markers (in spite of not being altogether cardiac-specific), with wellestablished
roles in confirming acute myocardial infarction (AMI) and in monitoring reperfusion during thrombolytic
therapy following AMI.2

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 baseline levels within 48 to 72 hours.15 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 levels have been used in this context to assess reperfusion.

PRINCIPLE

Immunoenzymometric assay:

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-Insulin antibody.

Upon mixing monoclonal biotinylated 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:

$$^{Enz}\underline{Ab}_{(m)} + \underline{Ag}_{CK\text{-}MB.} + {}^{Btn}\underline{Ab}_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}\underline{Ab}_{(m)}\text{-}\underline{Ag}_{TSH}\text{-}{}^{Btn}\underline{Ab}_{(m)}$$

$^{Btn}\underline{Ab}_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)

$\underline{Ag}_{CK\text{-}MB.}$ = Native Antigen (Variable Quantity)

$^{Enz}\underline{Ab}_{(m)}$= Enzyme labeled Monoclonal Antibody (Excess Quantity)

$^{Enz}\underline{Ab}_{(m)}\text{-}Ag_{CK\text{-}MB.}\text{-}{}^{Btn}Ab_{(m)}$ = Antigen-Antibodies complex

k_a = Rate Constant of Association

k_{-a} = 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:

$^{Enz}\underline{Ab}_{(m)}\text{-}\underline{Ag}_{CK\text{-}MB.}\text{-}{}^{Btn}\underline{Ab}_{(m)} + \underline{Streptavidin}_{C.W.} \Rightarrow \underline{immobilized\ complex}$

$\underline{Streptavidin}_{C.W.}$ = Streptavidin immobolized on well
Immobilized 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 antigen concentration of an unknown can be ascertained.

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 required tests. 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

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 red-top venipuncture tube without additives. 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 two (2) 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, 0.100ml of the specimen is required.

REAGENTS AND MATERIALS PROVIDED:

1. **CK-MB Calibrators – 1.0 ml/vial (Lyophilized) [Icons A – F]**
   Six (6) vials of references for CK-MB antigen at levels of 0(**A**), 2.0(**B**), 4.0(**C**), 10.0(**D**), 40(**E**), and 100(**F**) ng/ml. Reconstitute each vial with 2.0ml 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 criteria from a highly purified reference preparation.
2. **Anti-CK-MB-HRP and Biotinylated monoclonal antibodies —13 ml/vial [Icon Ⓔ]**
   One (1) vial containing enzyme labeled affinity purified monoclonal mouse antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.
3. **Streptavidin Microplate -- 96 wells – [Icon⋓]**
   One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
4. **Wash Solution Concentrate – 20 ml [Icon ♠]**
   One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.
5. **Substrate A --7.0ml/vial – [Icon S^A]**
   One (1) bottle containing Luminol in buffer. Store at 2-8°C.
6. **Substrate B -- 7.0ml/vial – [Icon S^B]**
   One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.
8. **Product Insert:** (Instruction Booklet).

Required But Not Provided:

1. Pipette(s) capable of delivering 25µl and 100µl volumes with a precision of better than 1.5%.
2. Dispenser(s) 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. Micoplate luminometer.
5. Adjustable volume (200-1000µl) repeat dispenser.
6. Container(s) for mixing of reagents (see below).
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Storage container for storage of wash buffer.
12. Distilled or deionized water.
13. Quality Control Materials.

REAGENT PREPARATION:

1. **Wash Buffer**
   Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.
3. **Working Substrate Solution**
   Mix equal volumes of Solution 'A' and Solution 'B' in a clean container. Use within 60 minutes. For example, add 1ml of A and 1ml of B for two (2) eight well strips (A slight excess of solution is made. Discard the unused portion).

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 microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of the biotinylated/enzyme labeled antibodies to each well. **It is very important to dispense all reagents close to the bottom of the microwell.**
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap.
5. Incubate for 120 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 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) 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 two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells ***(see Reagent Preparation Section)***.
9. Incubate at room temperature for five (5) minutes.
10. Read the relative light units for 0.2-1.0 seconds per well with a microplate luminometer.

The results should be read within thirty (30) minutes of adding the substrate solution.

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

QUALITY CONTROL

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 test procedure performed. Quality control trends should be maintained to monitor batch to batch consistency.

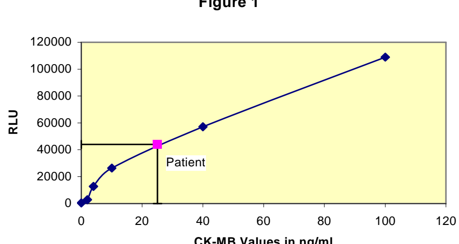
CALCULATION OF RESULTS

1. Record the RLU's obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLU's for each duplicate serum reference versus the corresponding CK-MB concentration in mIU/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 RLU of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (ng/ml)
Cal A	A1	406	413	0
	B1	421		
Cal B	C1	2744	2832	2.0
	D1	2921		
Cal C	E1	12931	12664	4.0
	F1	12397		
Cal D	G1	25944	26384	10
	H1	26824		
Cal E	A2	53935	57086	40
	B2	60238		
Cal F	C2	106907	108920	100
	D2	110934		
Ctrl 1	E2	3194	3269	4.03
	F2	3344		
Ctrl 2	G2	30957	32255	37.6
	H2	33554		
Patient 1	A3	11415	12010	22.3
	B3	12605		

Figure 1



*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Quality Control:

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

1. The RLU of calibrators 0 ng/ml should be ≤ 1000
2. The RLU of calibrators 100 ng/ml should be ≥ 70,000.
3. Four out of six quality control pools should be within the established ranges.

LIMITATIONS OF PROCEDURE

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
5. Highly lipemeic, hemolysed or grossly contaminated specimen(s) should not be used.
6. Patient samples with CK-MB concentrations above 10 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.
7. 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 smokers and non smokers, the smokers have a slightly higher base line.

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 Diagnostic Automation, Inc. 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

PERFORMANCE CHARACTERISTICS

A. Precision

The within and between assay precision of the CK-MB CIA Microplate Test System were determined by analyses on two different levels of pool 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	C.V.
Pool 1	20	0.82	0.07	8.53%
Pool 2	20	3.16	0.19	6.00%
Pool 3	20	8.11	0.52	6.40%

TABLE 3

Between Assay Precision* (Values in ng/ml)

SAMPLE	N	X	σ	C.V.
Pool 1	20	0.79	0.09	11.2%
Pool 2	20	3.31	0.22	6.61%
Pool 3	20	8.99	0.85	9.45%

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

B. Accuracy

The *Diagnostic Automation, Inc.* CK-MB Microplate CIA was compared with a predicate radioimmunoassay assay. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from 0.2 ng/ml – 11.8ng/ml). The total number of such specimens was 124. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	1.068	y = 0.2079 + 0.8036(x)	0.962
Reference	1.066		

Only slight amounts of bias between the CIA CK-MB 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.

C. Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.025 ng/ml.

D. Specificity

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

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