CE

AccuDiag™ CK-MB ELISA Kit

REF 1005-P1

IVD See External Label 2°C-8°C 96 Tests

CK-MB ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Peroxidase – Conjugated Competitive ELISA
Detection Range	0-400ng/ml
Sample	25 µL serum
Incubation Time	30 minutes
Shelf Life	18 Months from the manufacturing date
Specificity	96%
Sensitivity	0.182 ng/ml

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

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

SUMMARY AND EXPLANATION

Diagnostic Automation 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 altogether 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 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.[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 Heart Emergency Room (ER) Program[13] documented that serial testing for CK-MB isoenzyme (CK-MB, EC 2.7;3.2) 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 color.

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.

ASSAY 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-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 stearic hindrance, to form a

soluble sandwich complex. The interaction is illustrated by the following equation:

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

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{CK\text{-}MB}$ = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(m)}$ = Enzyme labeled MoAb (Excess Quantity)
$^{Enz}Ab_{(m)}$ - $Ag_{CK\text{-}MB.}$-$^{Btn}Ab_{(m)}$ = 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:

$$^{Enz}Ab_{(m)}\text{-}Ag_{CK\text{-}MB}\text{-}{}^{Btn}Ab_{(m)} + Strept_{C.W.} \Rightarrow \text{immobilized complex}$$

$Strep_{C.W.}$ = Streptavidin immobilized 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.

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. 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.

REAGENTS

Materials provided with the test kit

1. **CK-MB Calibrators – 1.0 ml/vial (Lyophilized) [A – F]**
   Six (6) vials of references for CK-MB antigen at levels of 0(**A**), 5.0(**B**), 25.0(**C**), 100.0(**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 seen with PAGE.
2. **CK-MB Enzyme Reagent – 13 ml/vial**
   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.
3. **Streptavidin Plate – 96 wells**
   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/vial**
   One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
5. **Substrate A – 7.0 ml/vial**
   One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.
6. **Substrate B – 7.0 ml/vial**
   One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.
7. **Stop Solution – 8.0 ml/vial -**
   One (1) bottle containing a strong acid (1N HCl). Store at 2-8°C.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.
Note 3: Above reagents are for a single 96-well microplate.

Materials required but not provided

1. Pipette(s) capable of delivering 25 µl and 50 µl 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 washers or a squeeze bottle (optional).
4. Microplate Reader with 405nm and 620nm wavelength absorbance capability. (The 620nm filter is optional)
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or micro plate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer
9. Storage container for storage of wash buffer.
10. Distilled or deionized water.
11. Quality control materials.

REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
2. **Working Substrate Solution – Stable for one year**
   Pour the contents of the 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.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature.
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 0.025 ml (25µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of the CK-MB Enzyme Reagent to each well. It is very important to dispense all reagents close to the bottom of the microwell.

Note: Use a multichannel pipet to quickly dispense the 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 two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instructions 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).

DO NOT SHAKE 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 450nm (using a reference wavelength of 620-630nm 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.

RESULTS

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

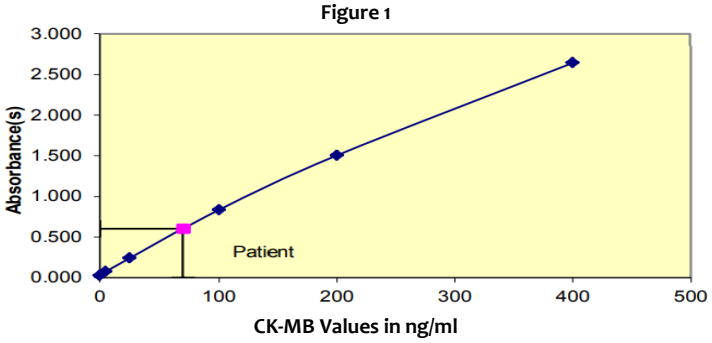
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CKMB concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of CK-MB for an unknown, locate the average absorbance 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 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 absorbance (0.136) intersects the dose response curve at (12.4 ng/ml) CK-MB concentration (See Figure 1).

Note: Computer data reduction software designed for DAI (ELISA) assays may also be used for the data reduction.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.022	0.022	0
	B1	0.023		
Cal B	C1	0.072	0.071	5
	D1	0.070		
Cal C	E1	0.243	0.236	25
	F1	0.230		
Cal D	G1	0.851	0.833	100
	H1	0.815		
Cal E	A2	1.503	1.504	200
	B2	1.505		
Cal F	C2	2.567	2.612	400
	D2	2.658		
Ctrl 1	E2	0.046	0.049	2.35
	F2	0.052		
Ctrl 2	G2	0.585	0.592	70.3
	H2	0.598		
Patient 1	A3	0.140	0.136	12.4
	B3	0.131		

Figure 1



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

Q.C. 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 < 0.075.
2. The absorbance (OD) of calibrators 'F' should be > 1.8.
3. Four out of six quality control pools should be within the established ranges.

PERFORMANCE CHARACTERISTICS

Precision

The within and between assay precision of the CK-MB DAI ELISA test system were determined by analyses on three different levels of pool control sera. The number (N) mean value (X), standard deviation (σ) and coefficient of variation (C.V.) 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	12.11	0.59	4.87%
Pool 3	20	58.10	3.74	6.44%

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

Sample	N	X	σ	C.V
Pool 1	20	0.86	0.09	10.4%
Pool 2	20	13.31	1.22	9.16%
Pool 3	20	52.52	2.84	5.45%

*As measured in ten experiments in duplicate.

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 0.182 ng/ml.

Accuracy
The CK-MB Diagnostic Automation, Inc. ELISA test system was compared with a predicate radioimmunoassay assay. 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 124. The data obtained is displayed in Table 4.

TABLE 4
Least Square

Method	Mean (x)	Regression Analysis	Correlation Coefficient
This Method (y)	**12.52**	**y =0.5477+0.9946(x)**	**0.971**
Reference (x)	**12.04**		

Only slight amounts of bias between the CK-MB DAI ELISA 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.

Specificity
The cross-reactivity of the CK-MB DAI ELISA 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.

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 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 DACD IFU may yield inaccurate results.
11. 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.
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.

INTERPRETATION

1. Measurements and interpretation of results must be performed by a skilled individual or 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 the test system 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 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, DACD 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.

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 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**

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 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.

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.

REFERENCES

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

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Date Adopted	**2022-09**
Brand Name	**AccuDiag™**
REF 1005-P1	**AccuDiag™ - CK-MB ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands** www.cepartner4u.eu
Revision Date: 2021-06-02	