Datasheet for ABIN996908

Creatine Kinase MB ELISA Kit

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Overview

Quantity:	96 tests
Target:	Creatine Kinase MB (CKM)
Reactivity:	Human
Method Type:	Competition ELISA
Detection Range:	0-400 ng/mL
Minimum Detection Limit:	0 ng/mL
Application:	ELISA

Product Details

Purpose:	The Quantitative Determination of Circulating Creatinine Kinase (MB-Isoform) Concentrations in Human Serum by a Microplate Immunoenzymometric assay.
Sample Type:	Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	96%
Sensitivity:	0.05 ng /mL
Material not included:	1. Pipette(s) capable of delivering 25 mL and 50 mL volumes with a precision of better than 1.5 %. 2. Dispenser(s) for repetitive deliveries of 1. 00 mL and 1. 00 mL volumes with a precision of better than 1. % (Optional) 3. Microplate washers or a squeeze bottle (optional).

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4. Microplate Reader with 405 nm and 620 nm wavelength absorbance capability. (The 620 nm 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.

Target Details

Target:	Creatine Kinase MB (CKM)
Abstract:	CKM Products
Background:	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). 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. 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. 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 documented that serial testing for CK-MB isoenzyme

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(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.

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Comment:	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 - follow the performance of the supplied reagents. Pertinent statistical methods should be employed - ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used - determine the reason for the variations.
Sample Volume:	25 µL
Assay Time:	1 h
Plate:	Pre-coated
Sample 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. Centrifuging serum samples before a complete forms may result in the presence of fibrin. To prevent erroneous results due to the presence of

	fibrin make sure that complete clot formation has taken place prior to the centrifugation of the samples. 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, 0.100 mL of the specimen is required
Assay Procedure:	Before proceeding with the assay, bring all reagents, serum references and controls to room temperature. 1. Format the microplates' wells for each serum reference, 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 (25 mL) of the appropriate serum reference, control or specimen into the assigned well. 3. Add 0.100 mL (100 mL) of the CK-MB-Enzyme Reagent to all the 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. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap. Incubate for 15 minutes at room temperature (20-25 °C). Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper. Add 300 mL 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. Add 0.100 mL (100 mL) of working substrate solution to all wells (see Reagent Preparation Section). 9. Incubate at room temperature for fifteen (15) minutes. 10. Add 0.050 mL (50 mL) of stop solution to each well and mix gently for 15-20 seconds. Read the absorbance in each well at 450 nm (using a reference wavelength of 620-630 nm 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.
Calculation of 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 (1
2.4 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 Al 0.022 0.022 0 Bl 0.023 Cal B Cl 0.072 0.071 5 Dl 0.070 Cal C El 0.243 0.236 25 Fl 0.230 Cal D Gl 0.851 0.833 100 hi 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 .0136 1
2.4 B3 0.131 cc 11C.)
A. 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 (sigma) 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 sigma C.V Pool 1 20 0.82
0.07 8.53 % Pool 2 20 1
2.11 0.59
4.87 % Pool 3 20 58.10
3.74

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	6.44 % TABLE 3 Between Assay Precision* (Values in ng/mL) Sample N X sigma C.V Pool 1 20 0.86 0.09 10.4 % Pool 2 20 1 3.31 1.22 9.16 % Pool 3 20 5 2.52 2.84 5.45 % *As measured in ten experiments in duplicate. B. 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 in concordance with the published literature the following ranges have been assigned.
Restrictions:	For Research Use only

Handling

Storage:	4 °C
Expiry Date:	18 months