Datasheet for ABIN649048

Creatine Kinase MB Isoenzyme Type II ELISA Kit

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Overview

Quantity:	96 tests
Target:	Creatine Kinase MB Isoenzyme Type II
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	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 stearic hindrance, to form a soluble sandwich complex. After equilibrium is attained, the antibodybound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibodybound 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.
Analytical Method:	Quantitative
Detection Method:	Colorimetric

Product Details

Components:	A. CK-MB Calibrators (1. 0 ml/vial) (Lyophilized). Six vials of references for CK-MB antigen 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 greater than99% purified preparation as seen with PAGE. B. CK-MB Enzyme Reagent (13 ml/vial). One vial containing enzyme labeled affinity purified antibody and biotin labeled monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C. C. 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. D. Wash Solution Concentrate (20 ml). One vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C. E. Substrate A (7. 0ml/vial). One bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. F. Substrate B (7. 0ml/vial). One bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C. G. Stop Solution (8. 0ml/vial). One bottle containing a strong acid (1N HCl). Store at 2-30°C. H. Product Instructions: Note 1: Do not use reagents beyond the kit expiration date. Note 2: Opened reagents are stable for 60 days when stored at 2-8°C. Note 3: Above reagents are for a single 96-well microplate.
Material not included:	1. Pipette (s) capable of delivering 25µl and 50µl volumes with a precision of better than 1. 5%. 2. Dispenser(s) for repetitive deliveries of 0. 100ml and 0. 350ml volumes with a precision of better than 1. 5% (optional). 3. Microplate washer or a squeeze bottle (optional). 4. Microplate Reader with 450nm and 620nm wavelength absorbance capability (The 620nm filter is optional). 5. Absorbent Paper for blotting the microplate wells. 6. Plastic wrap or microplate 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 Isoenzyme Type II
Alternative Name:	CK-MB (CK-2) (Creatine Kinase MB Isoenzyme Type II Products)
Background:	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 altogether cardiac-specific), with well established roles in confirming acute myocardial infarction (AMI) and in monitoring reperfusion during thrombolytic therapy following AMI. 2In 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. Intended Use: The Quantitative Determination of Circulating Creatinine Kinase (MB-Isoform) Concentrations in Human Serum by a Microplate Immunoenzymometric assay.

Application Details

Application Notes:	Precautions: 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.
Plate:	Pre-coated
Reagent Preparation:	1. Wash Buffer: Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27 °C for up to 60 days. 2. Working Substrate Solution: 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: Do not use the working substrate if it looks blue.
Sample Collection:	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. Samples may be refrigerated at 2-8°C for a maximum period of five 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. 050 ml of the specimen is required.
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. 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). 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). Note: Computer data reduction software designed for IEMA (ELISA) assays may also be used for the data reduction.
Restrictions:	For Research Use only

Handling

Handling Advice:	Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°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. 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 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two additional times for a total of three 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 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 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 30 minutes of adding the stop solution. Note: Always add reagents in the same order to minimize reaction time differences between wells.
Storage:	4 °C/-20 °C