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CKMB ELISA Kit

Name	CKMB ELISA Kit
INTENDED USE	For the quantitative determination of CK-MB concentration in human serum.
SUMMARY AND EXPLANATION	Creatine Kinase (CK-MB) is the enzyme being used as the definitive serum marker for the diagnosis or exclusion of acute myocardial infarction (AMI).The determination of CK-MB mass has proven to be more specific for myocardial necrosis than the long-standing CK-MB activity and CK-MB inhibition assays. CK-MB, released after AMI, is detectable in blood as early as 3-4 hours after the onset of symptoms, and remains elevated for approximately 65 hours post infarct 8-9. CK-MB mass levels are reportedly 50% diagnostic for AMI after 3 hours and > 90% diagnostic at 6 hours 10. Such accuracy makes CK-MB mass determinations useful in confirming AMI in patients presenting to the ER with non-diagnostic ECGs > 6 hours after the onset of symptoms.
PRINCIPLE OF THE TEST	The CK-MB ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the CK-MB molecule is used for solid phase immobilization (on the microtiter wells). A goat anti-CK-MM antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CK-MB molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 1 hour incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color.

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The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of CK-MB is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

MATERIALS PROVIDED	96 Tests
Antibody-Coated Microtiter Plate	12x8x1
Liquid CK-MB Standards containing:0,7.5,15,50,100, & 200 ng/ml CK-MB. 1.0 ml for each standard dose. Store at -20°	1ml
Enzyme Conjugate Reagent, (Ready to Use)	22 ml
TMB Reagent (one-step)	11ml
Stop Solution	11ml
Wash Concentrate 20x: 1 Bottle	25 ml

MATERIALS NOT PROVIDED

Precision pipettes: 0.02 ml, 0.1 ml, 0.2 ml, and 1 ml.
Disposable pipette tips.
Distilled water.
Vortex mixer or equivalent.
Absorbent paper or paper towel.
Graph paper.
Microtiter plate reader.

STORAGE AND STABILITY

1. CK-MB standards are not stable at room temperature. The
standards are stable at 2-8ºC for at least 7 days. It is recommended that the standards are aliquoted and stored at
-20ºC or below.
2. Unopened test kits should be stored at 2-8C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date

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	shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.
WARNINGS AND PRECAUTIONS	Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984 Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. It is recommended that serum samples be run in duplicate. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
SPECIMEN COLLECTION HANDLING	Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.
REAGENTS PREPARATION	All reagents should be brought to room temperature (18-25C) before use. Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or demonized water. Store at room temperature (18-26 C).
ASSAY PROCEDURE	Secure the desired number of coated wells in the holder.

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	Dispense 20 l of standard, specimens, and controls into appropriate wells. Dispense 200 l of Enzyme Conjugate Reagent to each well. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup. Incubate at room temperature (18-25C) for 60 minutes. Remove liquid from all wells. Wash wells three times with 300 L of 1X wash buffer. Blot on absorbance paper or paper towel. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets. Dispense 100 l of TMB Reagent into each well. Gently mix for 5 seconds. Incubate at room temperature for 20 minutes. Stop the reaction by adding 100 l of Stop Solution to each well. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.
CALCULATION OF RESULTS	Calculate the average absorbance values (A450) for each set of reference standards, control, and samples. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis. Using the mean absorbance value for each sample, determine the corresponding concentration of CK-MB in ng/ml from the standard curve. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculation.
EXAMPLE OF STANDARD CURVE	Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against CK-MB

concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

LIMITATIONS OF THE TEST

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

EXPECTED VALUES

Normal range for CK-MB reported by various literatures is between 0-9.0 ng/ml. It is recommended that each laboratory establish its own normal range. The minimum detectable concentration of CK-MB by this assay is estimated to be 2.5 ng/ml.

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