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Product Information

CK-MB ELISA kit

Catalog Number: EA100892
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The CK-MB ELISA Kit is intended for the quantitative determination of CK-MB concentration in human serum.

Background

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. 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.

Components

MATERIALS PROVIDED	96 Tests
1. Antibody-Coated Microtiter Plate	12x8x1
2. 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°	1 ml

3. Enzyme Conjugate Reagent, (Ready to Use)	22 ml
4. TMB Reagent (one-step)	11 ml
5. Stop Solution	11 ml
6. Wash Concentrate 20x: 1 Bottle	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

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.

Reagent Preparation

Prepare 1X Wash buffer by adding the Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- 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 standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

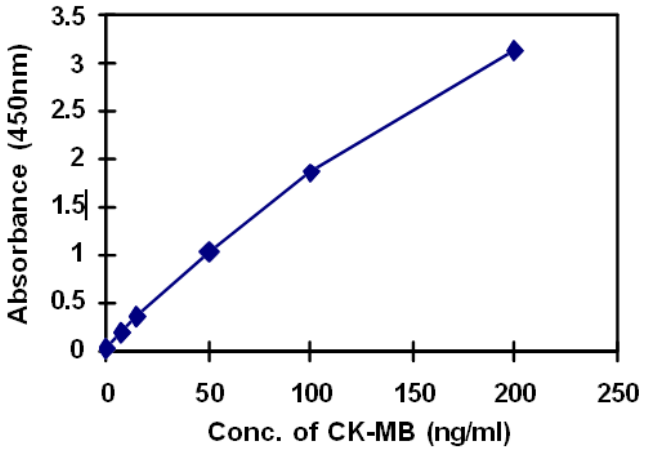
1. Secure the desired number of coated wells in the holder.
2. Dispense 20 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 200 µl of Enzyme Conjugate Reagent to each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 60 minutes.
6. Remove liquid from all wells. Wash wells three times with 300 µl of 1X Wash buffer. Blot on absorbance paper or paper towel.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
9. Incubate at room temperature for 20 minutes.
10. Stop the reaction by adding 100 µl of Stop Solution to each well.
11. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

Calculation of Results

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. 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.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CK-MB in ng/ml from the standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculation.

Example of a 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.



Expected Values and Sensitivity

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.

References

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