Product Manual

Human CK- MB ELISA Kit (Creatine Kinase- MB)

Catalog Numbers

PRB- 5047	96 assays
PRB- 5047- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Creatine kinase (CK), also known as creatine phosphokinase, catalyses the conversion of creatine to phosphocreatine, utilizing ATP. Phosphocreatine (PCr) serves as an energy reserve in tissues which consume ATP rapidly (muscle, brain, heart), making CK an important enzyme. There are 3 cytosolic isotypes of creatine kinase: CK-MM, CK-BB, and CK-MB. The CK-MM variety is primarily found in skeletal muscle, while CK-BB is mainly associated with brain and smooth muscle tissue. The CK-MB isoenzyme is predominantly found in the myocardium (heart muscle). Following a heart attack (myocardial infarction), damaged cells release CK-MB into the blood; these elevated CK levels can be seen 4-8 hours post-infarction and remain elevated for a few days. This makes creatine kinase a useful biomarker for assessing damage to CK-rich tissues, as in cases of rhabdomyolysis (severe muscle breakdown), acute kidney injury, and heart attack.

Cell Biolabs' CK-MB ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the human CK-MB protein. The kit has detection sensitivity limit of 350 pg/mL CK-MB. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and CK-MB samples.

Note: This kit is CK-MB isotype specific (Figure 2).

Assay Principle

An anti-CK-MB coating antibody is adsorbed onto a microtiter plate. CK-MB protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-CK-MB antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin-enzyme conjugate is added and binds to the biotinylated anti-CK-MB antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of CK-MB present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified CK-MB and sample concentration is then determined.

Related Products

1. PRB-5033: Human Alpha 2 Macroglobulin ELISA Kit
2. PRB-5034: Human Alpha 1 Antitrypsin ELISA Kit
3. PRB-5038: Human Beta 2 Microglobulin ELISA Kit
4. PRB-5039: Human Haptoglobin ELISA Kit
5. PRB-5041: Human Ceruloplasmin ELISA Kit
6. PRB-5044: Human Alpha 1 Antitrypsin ELISA Kit
7. PRB-5048: Human D-Dimer ELISA Kit
8. PRB-5050: Human Troponin I ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-CK-MB Antibody Coated Plate (Part No. 50471B): One strip well 96-well plate.
2. Biotinylated Anti-CK-MB Antibody (1000X) (Part No. 50472D): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human CK-MB Standard (Part No. 50473D): One 100 µL vial of 10 µg/mL human CK-MB.

Materials Not Supplied

1. CK-MB Sample: serum, plasma, lysate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store CK-MB Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-CK-MB Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-CK-MB Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of CK-MB Standard in the concentration range of 10 ng/mL – 0.156 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	10 µg/mL Human CK-MB Standard (µL)	Assay Diluent (µL)	CK-MB (ng/mL)
1	4	3996	10
2	500 of Tube #1	500	5
3	500 of Tube #2	500	2.5
4	500 of Tube #3	500	1.25
5	500 of Tube #4	500	0.625
6	500 of Tube #5	500	0.313
7	500 of Tube #6	500	0.156
8	0	500	0

Table 1. Preparation of CK-MB Standard

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Add 100 µL of CK-MB sample or standard to the Anti-CK-MB Antibody Coated Plate. Each CK-MB sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
4. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-CK-MB Antibody to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
9. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.
11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical CK-MB ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

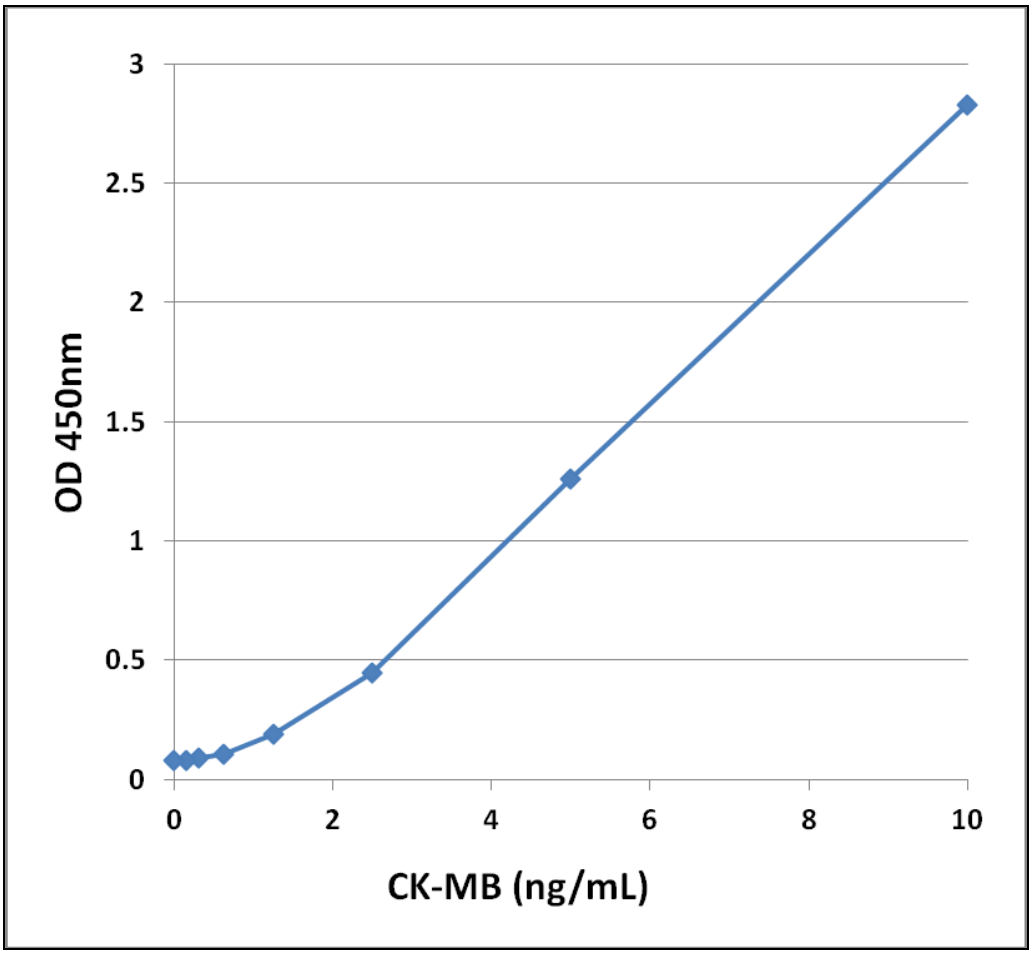

Figure 1: CK-MB ELISA Standard Curve

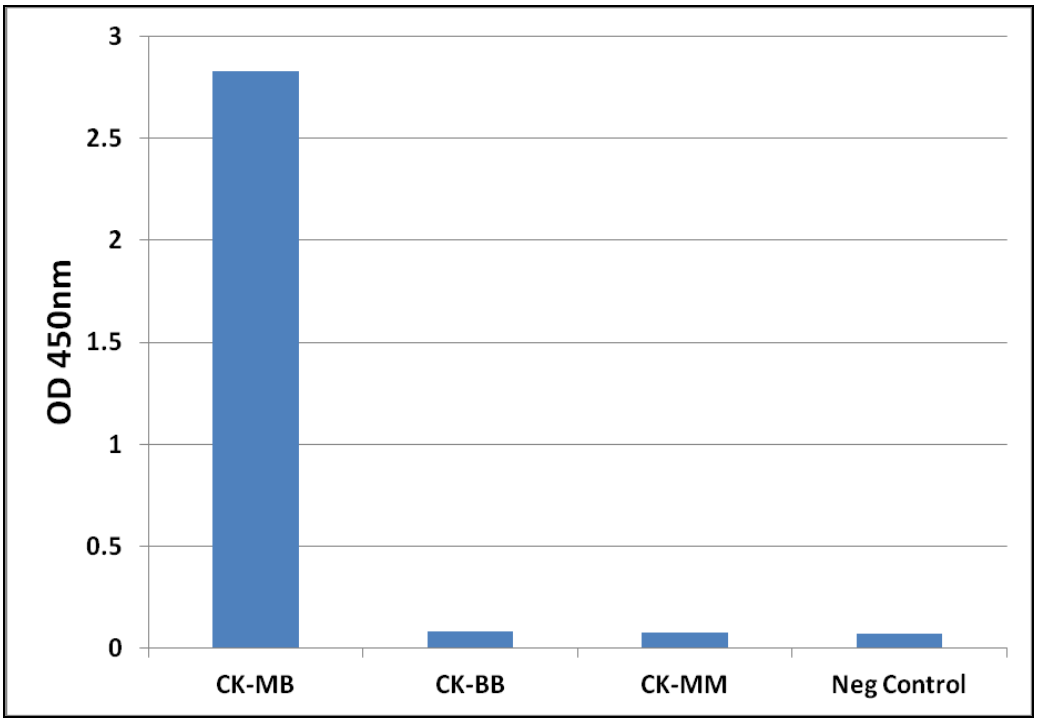

Figure 2: CK Isotype Specificity. Purified CK-MB, CK-BB, and CK-MM samples were prepared at 5 ng/mL and tested according to the Assay Protocol.

References

1. Gibler, W., et al. (1990) *Ann. Emerg. Med*. **19**:1359-1366.
2. Hetland, O., K. Dickstein (1996) *Scand. J. Clin. Lab. Invest.* **56**:701-713.
3. Penttila, K., et al. (2002) *Clin. Biochem*. **35**:647-653.

Warranty

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