

## STORAGE OF TEST KIT AND INSTRUMENTATION

### Enzyme Immunoassay for the Quantitative Determination of Creatine Kinase (CK-MB) in Serum

Catalog Number: 11210

FOR RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROCEDURES

STORE AT 2 TO 8°C

### PRINCIPLE OF THE TEST

The CK-MB ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay<sup>13-14</sup>. 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.

### REAGENTS

#### **Materials provided with the kit:**

- Antibody-coated microtiter plate with 96 wells.
- Liquid CK-MB standards containing; 0, 7.5, 15, 50, 100, and 200 ng/ml CK-MB. 1.0 ml for each standard dose. Store at -20°C or below.
- Enzyme Conjugate Reagent, 22 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

1. CK-MB standards are not stable at room temperature. The standards are stable at 2-8 °C for at least 7 days. It is recommended that the standards are aliquoted and stored at -20° C or below.
2. Unopened test kits should be stored at 2-8°C 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 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.

### REAGENT PREPARATION

All reagents should be brought to room temperature (18-25°C) before use.

### ASSAY PROCEDURE

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 the incubation mixture by emptying plate content into a waste container.
7. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
13. Read the optical density at 450 nm with a microtiter plate reader ***within 15 minutes.***

### CALCULATION OF RESULTS

1. Calculate the average absorbance values ( $A_{450}$ ) 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.

OxisResearch™

1499 Rollins Road  
 Burlingame, CA 94010 U.S.A.  
 E-mail: info@aoxre.com  
 Telephone: 650-289-8908  
 www.aoxre.com  
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### 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.

CK-MB (ng/ml)	Absorbance (450 nm)
0	0.047
7.5	0.206
15	0.366
50	1.028
100	1.856
200	3.128

