

## CREATINE KINASE (CK - MB) IMMUNO INHIBITION

### INTENDED USE:

This reagent kit is intended for "*in vitro*" quantitative determination of Creatine Kinase - MB) activity in serum based upon IFCC and DGKC recommendations.

### CLINICAL SIGNIFICANCE:

CK-MB is an enzyme formed by the association of two subunits from muscle (M) and nerve cells (B). CK-MB is usually present in serum at low concentration; it increases after an acute infarct of myocardium and later descends at normal levels. Also is increased, rarely, in skeletal muscle damage. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

### PRINCIPE:

This procedure involves measurement of Creatine kinase (CK) activity in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of CKMB while not affecting the B subunit of CK-MB and CK-BB. Then the CK method is used to quantitatively determine CK-BB activity. CK catalyses the reaction between creatine phosphate and ADP, giving creatine and ATP. ATP and glucose in the presence of G6PDH oxidises, and reduces NAD to NADH. The rate of NADH formation is determined photometrically at 340 nm & is directly proportional to CK-BB activity. The CK-MB activity is calculated by multiplying CKBB x 2.

### REAGENT COMPOSTION:

Reagent 1: Enzyme Reagent 1  
 Reagent 2: Enzyme Reagent 2

### MATERIALS REQUIRED BUT NOT PROVIDED:

-Clean & Dry Glassware.  
 -Micropipettes & Tips.  
 -Colorimeter or Bio-Chemistry Analyzer.

### SAMPLES:

Serum free of hemolysis. Heparin or EDTA plasma.

### WORKING REAGENT PREPARATION & STABILITY:

Mix 4 Volume of Enzyme Reagent 1, with 1 Volume of Reagent Enzyme 2. Working Reagent is stable for 30 days at 2-8°C.

### GENERAL SYSTEM PARAMETERS:

Reaction type	Kinetic Reaction
Wave length	340 nm
Light Path	1 Cm
Reaction Temperature	37°C
Blank/Zero Setting	With Distilled Water
Reagent Volume	1ml
Sample Volume	50 µl
Lag/Delay Time	300 Sec.
Read Time	120 Sec.
Interval Time	60 Sec.
Factor	6752
Low Normal at 37°C	0 U/I
High Normal at 37°C	24 U/I
Linearity	1500 U/I
Max. $\Delta$ Abs/Min	0.222

### ASSAY PROCEDURE:

<b>Working Reagent</b>	<b>1000 µl</b>
<b>Sample</b>	<b>50 µl</b>

Mix and after 300 second incubation, measure the change in absorbance every minute during 2 minutes at 37°C.

Determine the  $\Delta A/min$ .

### CALCULATION:

Creatine Kinase - MB (CK-MB) activity (U/I) =  $\Delta A/min$ . X 6752

### LINEARITY

Reagent is linear up to 1500 U/I  
 Dilute the sample appropriately and re-assay if Creatine Kinase - MB (CK-MB) activity exceeds 1500 U/I or AAbs/min Exceeds 0.222. Multiply result with dilution factor.

### REFERENCE NORMAL VALUE:

0-24 U/I

### QUALITY CONTROL:

For accuracy it is necessary to run known controls with every assay.

### LIMITATION & PRECAUTIONS:

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagent during assay process.
5. Use clean glassware free from dust or debris.

### BIBLIOGRAPHY:

Young et al., Clin. Chem., 21:10 (1975)