BIOGENIX
INC. PVT. LTD.

CK-MB

Creatine Kinase-Muscle/Brain (CK-MB)
(Immunoinhibition method)

INTENDED USE:

CK-MB in vitro assay for the quantitative determination of isoenzyme Creatine Kinase-MB (CK-MB) in human serum.

INTRODUCTION:

Creatine Kinase exists as dimeric molecules composed of M and B subunits that form the isoenzymes MM, MB, and BB. The subunits M and B are immunologically distinct. CK-MM and CK-MB are distributed primarily in the skeletel muscle and heart muscle, respectively, while CK-BB is present mainly in the brain and in tissues composed of smooth muscle. Following acute myocardial infarction, CK-MB activity increases significantly and this elevation is highly specific for the laboratory diagnosis of myocardial infarction. Although total CK activity usually increases following myocardial infarction, in some patients only the CK-MB activity increases, while the total CK remains in the normal range. In this procedure CK activity is measured in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB, while not affecting the B subunit activity of CK-MB and CK-BB. Due to negligible concentrations of CK-BB in the circulation, the remaining activity, multiplied by a factor of 2, represents the activity of the CK-MB isoenzyme.

PRINCIPLE OF THE METHOD:

CK-MB, Serum sample is incubated with CK-MB reagent containing Ab specific to CK-M sub unit which completely inhibits the CK-M monomer. The activity of CK-B which is not inhibited by the Ab, is than measured by the following reaction

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\text{CK-B}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{Glucose-6-phosphatase} + \text{ADP}$$

$$\text{Glucose-6-phosphatase} + \text{NADP}^+ \xrightarrow{\text{G-6-PDH}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^+$$

REAGENTS COMPOSITION:

Reagent 1	• N-acetylcysteine (NAC) : 12gm/L • NADP : 2 gm/L • DAPP : 0.050 gm/L
Reagent 2	• Imidazole : 50 gm/L • Creatinine Phosphate : 30 gm/L • Dextrose : 30 gm/L • ADP-K : 15 gm/L • Anti CK-MM Ab :12 ml/L

EQUIPMENTS NEEDED BUT NOT PROVIDED:

- Biochemistry Analyzer, Spectrophotometer or colorimeter measuring at 340 nm.
- Calibrated micropipettes with variable volume, range volume 5-1000 µl.
- Dry thermostat for 37± 0.1 ºC.

REAGENT PREPARATION:

Working reagent to be prepare as 4 volume of reagent-1, mix with 1 volume of reagent-2.
This working reagent is stable for 15 Day at 2-8°C.

STORAGE AND STABILITY:

- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C.
- Protected from light and contaminations during their use.
- Do not use reagents:
  1. After the expiration date.
  2. Signs of reagent deterioration:
  3. Presence of particles and turbidity.
  4. Working Reagent exceeds the absorbance 0.700 at 340 nm against distilled water.

COLLECTING AND HANDLING OF SPECIMENS:

- Serum and Plasma is the preferred Specimen.
- Clear Unhemolysed serum is the recommended specimen sample.
- Serum CK appears stable for 3 days at 2-8°C. It is recommended that specimens be assayed immediately after collection.

ASSAY PROCEDURES:

Being the CK-MB all reagents to room temperature before use. Pipette in to test tube and test as fellow**:**

Reagents	Test
Working Reagent	1.0 ml
Specimen	50 µl

Mix well and aspirate. After the initial delay of 300 seconds, record the absorbance of the test at an interval of 30 seconds for the next 90 seconds at 340 nm. Determine the mean change in the absorbance and calculate the result.

CALCULATION:

Activity of CK-MB (IU/L) = ΔAbs. /min. X 6752

SYSTEM PARAMETERS:

Reaction Type (Mode)	:	Kinetic
Reaction Slope	:	Increasing
Wave Length	:	340 nm
Flow Cell Temperature	:	37 ˚C
Reagent volume	:	1000 µl
Sample volume	:	50 µl
Delay Time	:	240 sec
Measuring Time	:	60 sec
Factor	:	6752
Linearity	:	2000 IU/L
Units	:	IU/L
Low Normal	:	0 IU/L
High Normal	:	24 IU/L

NOTES:

1. Do not use recycled plastic tubes as they react with TBHBA chromogen leading to the false results.
   Always use soap and glycerol free glass tubes.
2. Contamination of reagents must be avoided. After use all the reagents must be immediately stored back at 2-8^{0}C.
3. Re-plug the CK-MB reagents vial after use.
4. Contamination by soap or glycerol will affect the assay.
5. For sample values higher than 2000 IU/L, dilute the sample with normal saline and multiply the result with appropriate dilution factor.
6. As with all the diagnostic procedure, the physician should evaluate the data obtained by the use of this kit in light of other clinical information.

QUALITY CONTROL:

To ensure adequate quality control, it is recommended that each run includes assayed normal and abnormal controls. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES:

Sample Type	At 37˚C

These values are for guidance purpose; each laboratory should establish its own reference range, according to its own geographic area.

PERFORMANCE CHARACTERISTICS:

Measuring range (Linearity):

The assay is linear up to 2000 IU/L

If the results obtained were greater than 2000 IU/L, dilute the sample to 1/2 with NaCl 9 gm/L and multiply the result by 2.

SENSITIVITY:

Lower detection limit 1 IU/L

ACCURACY:

Results obtained using the reagent compared well with other commercial reagents.

PRECISION:

N= 80	Intra Run		Inter Run	
	Control sample 1	Control sample 2	Control sample 1	Control sample 2
Mean (IU/L)	17	194	17	194
SD	0.69	1.04	0.86	1.76
CV%	4.03	0.54	5.05	0.90

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES:

No interference for mentioned concentrations.

REFERENCES:

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