BIOLABO
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CK-MB Isoenzyme Immunoinhibition Method

Reagent for quantitative determination of CK-MB isoenzyme (CK-2) of creatine kinase [EC 2.7.3.2] in human serum.

I REF K1217	**R1** 2 x 16 mL	**R2** 1 x 8 mL	
I REF K2217	**R1** 2 x 32 mL	**R2** 2 x 8 mL	
I REF K4217	**R1** 2 x 40 mL	**R2** 1 x 20 mL	
REF 95506	Calibrator enclosed in the Kit	**R1** 1 x 2 mL	**R2** 1 x 5 mL

TECHNICAL SUPPORT AND ORDERS
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Latest revision: www.biolabo.fr

CE

IVD

Made In France

I: corresponds to significant modifications

I INTENDED USE

This reagent is designated for professional use in laboratory (automated method).
It allows the quantification of CK-MB isoenzyme (CK-2) of creatine kinase [EC 2.7.3.2] in human serum.

I GENERALITIES (1)

Creatine kinase is a dimeric enzyme composed of two subunits which combine to form 3 distinct CK isoenzymes: CK-BB (CK-1), CK-MB (CK-2) et CK-MM (CK-3).

CK-MM is the main form in skeletal muscle. CK-BB is found in brain and smooth muscle. CK-MB is found in high level in myocardium (CK-MB is between 10 and 20 % of total CK activity) and in lesser amount in skeletal muscle (CK-MB is lesser than 2 % of total CK activity).

In absence of disease, most CK activity in serum is due to CK-MM. Acute myocardial infarction will result in increased CK-MB isoform circulating in serum. It begins between 4 and 6 h after the attack, then peaks between 12h and 24 h and returns to normal within 48 h.

PRINCIPLE (4) (5)

CK-NAC modified reagent contains a polyclonal antibody (specific to the CK-M monomer) which so completely inhibits CK-MM activity and one half of CK-MB activity.
Only the activity of the non-inhibited B monomer subunit, representing half of the CK-MB activity, is measured. The method assumes that CK-BB activity in the specimen is essentially zero.

1) Creatine phosphate + Mg-ADP $\xrightarrow{\text{B monomer subunit of CK-MB}}$ Creatine + ATP

2) D-Glucose + ATP $\xrightarrow{\text{HK}}$ ADP + G-6-Phosphate

3) G-6-Phosphate + $NADP^+$ $\xrightarrow{\text{G6PDH}}$ 6-Phosphogluconate + NADPH + H^+

The increase in absorbance measured at 340 nm is proportional to the CK-MB activity in the specimen.

REAGENTS

R1 CKM	Reagent 1	
Imidazole Acetate, pH 6,7	125	mmol/L
D-Glucose	25	mmol/L
N-Acetyl-L-cysteine	25	mmol/L
NADP	2	mmol/L
Magnesium Acetate	12,5	mmol/L
EDTA	2,02	mmol/L
HK (Hexokinase)	> 6800	UI/L

R2 CKM	Reagent 2	
Imidazole Acetate, pH 6,7	125	mmol/L
ADP	15,2	mmol/L
AMP	25	mmol/L
AP5A	103	µmol/L
G-6-PDH	> 8800	UI/L
Creatine Phosphate	250	mmol/L

Polyclonal antibody to the human CK-M (Inhib. 2000 IU/L at +37°C)
Reagents R1 and R2 are classified as dangerous.

Danger Repro. 1B: H360 - May damage fertility or the unborn child

P201: Obtain special instructions before use, P202: Do not handle until all safety precautions have been read and understood, P308+P313: IF exposed or concerned: Get medical advice/attention, P405: Store locked up, P501: Dispose of contents/container in accordance with dangerous waste regulations. Classification due to Imidazole < 1%. For more details, refer to Safety Data Sheet (MSDS)

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

I Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Ready for use (ratio 4:1)

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, reagents are stable when stored and used as described:

Unopened:
- Until expiry date stated on the label.

Once opened:
- Separated reagents are stable at least 21 days.
- Discard any reagent if cloudy or if reagent blank at 340nm > 1.200.

SPECIMEN COLLECTION AND HANDLING (1) (2)

Unhemolysed serum. Avoid anticoagulants such as heparin, EDTA, citrate or fluoride. Protect from light and store in an airtight container to prevent loss of CO_2.

If myocardial infarction is suspected, it is recommended to collect patient after 6 hours, 12 hours and 24 hours. Minimum requested number of collects is two: 12 hours and 24 hours after symptoms appearance.

CK-MB activity in serum is stable for 4 to 8 h at room temperature, 1 to 2 days at 2-8°C, 1 month at –20°C.

LIMITS (3) (4) (5)

CK-BB: capable to interfere with the assay (rarely present in serum).

Atypical isoenzymes: possible interference with the assay (one form, a complex of CK-BB and immunoglobulin G, more frequently found in elderly women). The presence of atypical isoenzymes does not undermine the value of the assay as the enzymes pattern over time shows a steady state. In acute myocardial infarction, CK-MB values will raise and return to normal levels in 48 hours.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Biochemistry Clinical Analyzer Kenza One, Kenza 240TX/ISE or Kenza 450TX/ISE

EXPECTED VALUES (2)

CK-MB	At 37°C< 25 IU/L
CK-MB/CK (%)	a CK-MB ratio between 6 and 25% is consistent with acute myocardial infarction. In case of suspicion of myocardial infarction, CK-MB values rise and return to normal levels in 48 hours.

Each laboratory should establish its own normal ranges for the population it serves.

PERFORMANCES

Manual procedure, 37°C, 340 nm

Linearity Range: between 10 and 318 IU/L

Detection limit: 2 IU/L

Precision:

Within-run N = 20	*Level 1*	*Level 2*
Mean (IU/L)	33.7	166.5
S.D. IU/L	1.00	3.76
C.V. %	3.0	2.3

Between run N = 20	*Level 1*	*Level 2*
Mean (IU/L)	31.3	161
S.D. IU/L	1.19	3.47
C.V. %	3.8	2.2

Comparison with commercially available reagent (same method):
Study realized on sera:

y = 0.976 x + 0.269 r = 0.9995

Analytical sensitivity: approx. 0.00134 abs/min for 10 IU/L

Interferences:

Turbidity	No interference up to 8 mmol/L
Bilirubin	No interference up to 600 µmol/L
Hemoglobin	No interference up to 372 µmol/L
Glucose	No interference up to 700 mg/dL

Other substances may interfere (see § Limits)

Performances and stability data on Kenza ONE, Kenza 240TX/ISE, Kenza 450TX/ISE and Kenza One are available on request

CALIBRATION

- REF 95506 HDL LDL CK-MB calibrator traceable to Internal Masterlot

The calibration frequency depends on proper instrument functions and on the preservation of reagent

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

QUALITY CONTROL

- REF 95516 HDL LDL CK-MB Control Level 1
- REF 95526 HDL LDL CK-MB Control Level 2
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and reassay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

PROCEDURE

Refer to validated application of the Kenza Analyzer used

CALCULATION

The analyzer provides directly final result.
Refer to the instruction of use of Kenza analyzer.

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 664-667, 1185-1190.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 310-315*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-189 to 3-190*
(4) *Mattenheimer H. CK-MB Methods and clinical significance ; Proceedings of the CK-MB symposium, Philadelphia, 1981 ; 51-57*
(5) *Stein W. CK-MB methods and clinical significance; Proceedings of the CK-MB symposium, Philadelphia, 1981; 61-74.*
(6) *National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices. NCCLS, 1984, NCCLS Publication EP5-T*

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with