BIOLABO
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CK-MB Isoenzyme Immunoinhibition Method

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

REF 97217	**R1** 10 x 3 mL	**R2** 1 x 30 mL
REF 97317	**R1** 8 x 20 mL	**R2** 8 x 20 mL

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

CE

IVD

Made in France

I: corresponds to significant modifications

INTENDED USE

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

GENERALITIES (1)

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. This one increases between 4 and 6 hours following the beginning of the attack, then peaks between 12 and 24 hours and returns to normal within 48 hours.

PRINCIPLE (4) (5)

The 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.

The reaction scheme is as follows:

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+ $\xrightarrow{\text{G6-PDH}}$ 6-Phosphogluconate + NADPH + H^+

The increase in absorbance due to the conversion of $NADP^+$ into NADPH, measured at 340 nm, is proportional to the CK-MB activity in the specimen.

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.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Spectrophotomètre thermostaté ou analyseur de biochimie clinique
2. Equipement de base de laboratoire

REAGENTS PREPARATION

Vial R1: Use a non-sharp instrument to remove aluminium cap.
REF 97217: add promptly 3 mL of buffer into vial R1.
REF 97317: add promptly contents of vial R1 into vial R2.
Mix gently and wait for complete dissolution

REAGENTS COMPOSITION

R1 **CKMB SUBS**	Enzymes Substrate
Creatine Phosphate	30 mmol/L
D-Glucose	20 mmol/L
N-Acetyl-L-cystein	20 mmol/L
AMP	5 mmol/L
ADP	2 mmol/L
NADP	2 mmol/L
AP5A	10 µmol/L
G-6-PDH (Glucose-6-phosphate dehydrogenase)	> 2500 UI/L
HK (Hexokinase)	> 3000 UI/L

Before reconstitution: **Caution**
Skin Irrit. 2: H315 – Causes skin irritation
STOT SE 3: H335 – May cause respiratory irritation
Acute Tox. 4: H302+H312+H332 – Harmful if swallowed, in contact with skin, if inhaled
Eye Irrit. 2: H319 - Causes serious eye irritation

P280: Wear protective gloves/protective clothing/eye protection/face protection, P264 : Wash hands thoroughly after handling, P501: Dispose of contents/container in accordance with dangerous waste regulations.

For more details, refer to Safety Data Sheet (MSDS)

Classification due to: **Creatine phosphate 25 - < 50%**

R1 **CKMB BUF**	Buffer
Imidazole Acetate, pH 6,8 at 30°C	100 mmol/L
EDTA Na2	2 mmol/L
Magnesium Acetate	10 mmol/L
Surfactant, stabilizer	

Polyclonal antibody to the human CK-M: To inhibit up to 2000 IU/L CKM
Contains also stabilizers and non-reactive fillers.

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

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)

Once reconstituted: Working Reagent (R1+R2) is classified as contents of vial R2 (Buffer)

STABILITY AND STORAGE

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

Unopened,
- Until the expiry date stated on the label of the Kit.

Once opened:
- Reconstitute immediately vial R1.

Once reconstituted
- Transfer requested quantity and store in the original vial at 2-8°C.
- Working reagent is stable at leastfor 3 weeks when free from contamination.
- Discard any reagent if cloudy or if reagent blank at 340 nm is > 0.700.
- Don't use working reagent after expiry date stated on the label.

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

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.

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.

SPECIMEN COLLECTION AND HANDLING (1) (2)

Unhemolysed serum. Store at 2-8°C and away from light. Use an air-tight container to prevent the loss of CO_2.

Plasma is not recommended because anticoagulants as heparin, EDTA, citrate or fluoride interfere with the determination.

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 hours at room temperature, 1 to 2 days at 2-8°C, 1 month at –20°c.

LIMITES (1) (3) (4) (5)

Haemolysis: adenylate kinase and other intermediates of the reaction as ATP (adenosine triphosphate) or G_6P (glucose-6-phosphate) interfere with the assay.

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.

EXPECTED VALUES (2)

	30°C	37°C
CK-MB	< 16 IU/L	< 25 IU/L
CK-MB/CK (%)	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.

REFERENCES

(1) *TIETZ N.W. Textbook 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*

PERFORMANCES

On Kenza 240TX, 37°C, 340 nm

Linearity Range: between 11 and 800 IU/L

Detection limit: approx. 3 IU/L

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	22.7	44.9	170
S.D. IU/L	0.9	1.0	2.6
C.V. %	3.8	2.1	1.5

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	23.3	45.4	169.2
S.D. IU/L	1.1	1.3	3.1
C.V. %	4.7	2.8	1.9

Comparison studies with commercially available reagent:

Realised on human specimens (n=100) between 10 and 750 IU/L

y = 0.9684 x + 0.4074 r = 0.9994

Interferences:

Total bilirubin	Negative interference from 276 µmol/L
Direct bilirubin	No interference up to 443 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 980 mg/dL
Turbidity	No interference of the turbidity
Haemoglobin	Positive interference from 38 µmol/L

Other substances may interfere (see § Interferences)

On the board stability: 2 weeks

Calibration Stability: 7 days

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

PROCEDURE

Manual procedure

Let stand reagents and specimens at room temperature.

Pipette into a 1 cm path length thermostated cuvette:	
Reagent	1 mL
Bring to 37°C, then add:	
Specimen	50 µL
Mix. After 5 minutes at 340 nm, record ΔAbs/min every minute for 5 minutes. Calculate the mean of ΔAbs/min.	

1- Performances with manual procedure should be validated by user.
2- Kenza applications and other applications proposal are available on request.

CALCULATION

With Calibrator:

$$\text{CK MB Activity} = \frac{(\Delta\text{Abs/min) Specimen}}{(\Delta\text{Abs/min) Calibrator}} \times \text{Calibrator Activity}$$

With Theoretical Factor:

Activity (U/L) = ΔAbs/min x Factor

$$\text{Factor} = \frac{VR \times 1000}{6.3 \times VE \times P}$$

With: VR = Total reactional volume (mL)
VE = Specimen volume (mL)
6.3 = Molar extinction coefficient for NADPH at 340nm
P = Pathlength (cm).

Example. with manual Procedure (Pathlength 1 cm. 37°C. 340 nm):

IU/L = (ΔAbs/min) x 6667

$$\mu\text{Kat/L} = \frac{\text{UI/L}}{60}$$

Calibration factor considers that CK-MB is 2 times CK - B activity.