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

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CE

IVD IN VITRO DIAGNOSTIC

CLINICAL SIGNIFICANCE (1)

Creatine kinase is a dimeric enzyme composed of two subunits. The monomer subunit M and the monomer subunit B. These subunits 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 activity represents between 10 and 20 % total CK activity) and in lesser amount in skeletal muscle (CK-MB activity represents 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. 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)

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.

The reaction scheme is as follows:

1) Creatine phosphate + Mg-ADP —(B monomer subunit of CK-MB, HK)→ Creatine + ATP

2) D-Glucose + ATP —(G6-PDH)→ ADP + G-6-Phosphate

3) G-6-Phosphate + $NADP^+$ → 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

BIOLABO reagents are designated for professional, in vitro diagnostic use (do not pipette with mouth).

- 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 should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Vial R1 (Enzymes-Substrate): Use a non-sharp instrument to remove aluminium cap.

REF 97217: add promptly 3 mL of vial R2 (Buffer) into the contents of vial R1.

REF 97317: add promptly the contents of vial R1 (Enzymes-Substrate), into vial R2 (Buffer).

Mix gently and wait for complete dissolution (approximately 2 minutes).

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. REF 95516, 95526 HDL LDL CK-MB Controls (human origin)
3. REF 95506 HDL LDL CK-MB Calibrator (human origin)

REAGENTS COMPOSITION

SUBS	SUBSTRATE- ENZYMES	Attention
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:
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, P302+P352 : IF ON SKIN: Wash with soap and water, P304+P340 : IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing, P305+351+338 : IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing, 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%**

BUF	BUFFER	Danger
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:		Quantity which inhibits CK-M up to 2000 IU/L at +37°C

Contains also stabilizers and non-reactive fillers.

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.

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

Classification due to: **Imidazole < 1%**

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

STABILITY AND STORAGE

Store at 2-8°C, well recap in the original vial and away from light.

- When stored and use as described in the insert, unopened reagents are stable until expiry date stated on the label.
- Once opened and reconstituted, working reagent is stable at least for 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.

CALIBRATION

Results will depend on the accuracy of the instrument calibration, the time counting, the respect of reagent/specimen ratio and the temperature control.

- Human calibrator REF 95506 HDL LDL CK-MB (calibration value determined with validated statistical techniques and metrologically controlled instrument)
- Or use the theoretical calibration factor (§ **CALCULATION**)

QUALITY CONTROL

- REF 95516, REF 95526 HDL LDL CK-MB Controls
- 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. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, verify analysis parameters: Wavelength, temperature, specimen/reagent ratio, time counting, calibration factor.
4. If control is still out of range, use a new vial of reagent and reassay
5. 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.

INTERFERENCES (1) (3) (4) (5)

Results of tests realised on KENZA Series Analyzers are indicated at § Performances.

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. 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

PERFORMANCES at 37°C ON KENZA 240TX

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.

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. Read initial absorbance after 5 minutes at 340 nm and then record absorbance every minute during 5 minutes. Calculate absorbance change per minute (ΔAbs/min).	

Notes:

1- Performances and stability data's have been validated on KENZA 240TX and KENZA 450TX.

2-With Manual Procedure on Spectrophotometer and on other automated analyzers, performances and stability should be validated by user. Applications proposal are available on request.

CALCULATION

With Seric Muticalibrator:

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

With Theoretical Factor:

Activity (U/L) = ΔAbs/min x Factor

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

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 takes into account that CK-MB activity is 2 times CK - B activity.