CREATINE PHOSPHOKINASE FRACTION MB (CK-MB)

Determination of the MB fraction of creatinekinase in serum and plasma.

TEST SUMMARY

CK-MB consists of the subunits CK-M and CK-B. Specific antibodies against CK-M inhibits the complete CK-MM activity (main part of the total CK activity) and the CK-M subunity of CK-MB. Therefore only CK-B activity is measured, which is half of the del CK-MB.
The examined enzyme catalyses the hydrolysis of Creatine Phosphate forming ATP, which transform Glucose in Glucose-6-phosphate.
The Glucose-6-phosphate reacts with NADP converting it in NADPH caused an increase of absorbance at 340 nm.

SAMPLES

Serum is the preferred specimen. Plasma containing Heparin, EDTA, citrate, or fluoride may produce unpredictable reaction rates. CK activity in serum is unstable and is rapidly lost during storage. CK is inactivated both by bright daylight and by increasing specimen pH owing to loss of carbon dioxide; accordingly, specimens should be stored in the dark in tightly closed tubes. CK is susceptible to thermal denaturation; the degree of inactivation corresponds to the degree of temperature increase. Therefore, the serumspecimen should be chilled to 4°C as rapidly as possible after collection. A slight degree of hemolysis can be tolerated because erythrocytes contain no CK activity. However, moderately or severely hemolyzed specimens are unsatisfactory because anzymes and intermediates liberated from the erythrocytes may affect the lag phase and the side reactions occurring in the assay system.

REAGENTS

Reagent A: Imidazole buffer 0.1 M pH 6.70; Magnesium Acetate 10 mM; Glucose 20 mM; preservatives and stabilizers.

Reagent B: ADP 2 mM; AMP 10 mM; adenosinepentaphosphate 0.01 mM; NADP 2 mM; Exokinase (HK) ≥ 2000 U/l;Glucose-6-phosphate Dehydrogenase (G6P-DH) ≥ 1000 U/l; N-Acetyl-L-cysteine (NAC) 20 mM; EDTA 2 mM; Creatinephosphate 20 mM; specifical antibodies, preservatives and stabilizers.

REQUIRED MATERIALS BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Physiological solution.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GPL) guidelines.

REAGENTS PREPARATION

PROCEDURE STARTER SAMPLE

Add 6 ml of Reagent B to a vial of Reagent A. Work's reagent is stable 14 days at 2-8°C away from light sources

PROCEDURE STARTER REAGENT

Use reagents separately.
Stability: until expiration date on label away from light source.
Stability after first opening: ≥ 60 days.

PROCEDURE (STARTER SAMPLE)

Kind of analysis :	Kinetics (increasing)
Reading time:	1,2,3,4,5 minutes
Delay:	60 sec.
Wavelength:	340 nm
Temperature:	37°C
Lenghtpath:	1 cm
Zero:	Distilled water

REAGENTS	CUVETTE
Work reagent	1 ml
Preincubate at 37 °C at least for 5 minutes	
Sample	40 µl

PROCEDURE (STARTER REAGENTS)

Kind of analysis:	Kinetics (increasing)
Reading time:	1,2,3,4,5 minutes
Wavelength:	340 nm
Delay:	60 sec.
Temperature:	37°C
Pathelenght	1 cm
Zero:	Distilled Water

REAGENTS	CUVETTE
Reagent A Sample	1 ml 50 µl
Preincubate at 37 °C at least for 5 minutes	
Reagent B	250 µl

CALCULATION

Activity in U/l: ΔA/min x 8254

Activity in µkat/l: U/l x 0.0167

EXPECTED VALUES

Serum < 24 U/l (< 0.39 µkat/l)

Each laboratory should establish appropriate reference intervals related to its population.

NOTES

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control with control sera are available.

TEST PERFORMANCE

Precision

Intra-assay (n = 20)	Mean (U/l)	SD (U/l)	CV%
Sample 1	30.70	0.43	1.40
Sample 2	82.60	0.50	0.61

Inter-assay (n = 20)	Mean (U/l)	SD (U/l)	CV%
Sample 1	31.50	0.27	0.85
Sample 2	93.30	0.49	0.53

Sensibility/limit of detection

Method is able to discriminate up to 2 U/l.

Linearity

Method is linear up to 1000 U/l.
If ΔA/min is exceeded by 0.250, is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the results by 10.

Methods comparison

A comparison with a commercial available product gave the following results in a comparison on 75 samples:

CK-MB LTA = y
CK-MB competitor = x
n = 75

y = 0,97x + 3,78 U/l r = 0,998

Interferences

No interference was observed by the presence of:

lipids	≤ 2000 mg/dl
bilirubin	≤ 40 mg/dl

Hemoglobin interferences are possible.

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC01360	(120 TESTS)	
Reagent A	4 x 24 ml	(liquid)
Reagent B	1 x 24 ml	(liquid)

REFERENCES

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Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999. p. 617-721.
Wurzburg U, Hennrich N, Orth HD, Lang H. Quantitative determination of creatine kinase isoenzyme catalytic concentrations in serum using immunological methods. J. Clin Chem Clin Biochem 18977; 15:131-7.
4. Recommendations of the German Society for Clinical-Chemistry. Standarddization of methods for the estimation of enzyme activities in biological fluids: Standards method for the determination of creatine kinase acxtivity. J Clin Chem Clin Biochem 1977; 15:255-60.

MANUFACTURER

LTA s.r.l.
Via Milano 15/F
20060 Bussero (Milan) ITALY
Tel: ++39 02 95409034
Fax : ++39 02 95334185
e-mail: info@ltaonline.it
Website: http://www.ltaonline.it

SYMBOLS

IVD	Only for IVD use
LOT	Lot of manufacturing
REF	Code number
	Storage temperature interval
	Expiration date (year, month)
	Warning, read enclosed documents
	Read the directions
	Biological risk

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