

CK-MB FL

MB F120 CH

12 x 10 ml

SUMMARY OF TEST

Creatine kinase (CK) is an enzyme which is contained in heart, brain and skeletal muscles. Thus, an increase of circulating level of CK may be associated to myocardial infarct, acute cerebrovascular disease, trauma or diseases of skeletal muscles.

After a myocardial infarct, CK level begins raising between 4th and 6th hour after first acute symptoms, reaching the peak between 18th and 30th hour and coming back to normal values during the 3rd day.

CK is present in three different isoenzymatic forms, which could be separated by electrophoresis or column chromatography; each form is originated in different body tissues, paying off their diagnostic determinations.

CK exists in serum in dimeric forms as CK-MM, CK-MB, CK-BB and as macro-enzymes. Measurement of CK-MB is a quite specific test for detection of cardiac muscle damage and is therefore used for diagnosis and monitoring of myocardial infarction.

PRINCIPLE OF THE METHOD

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 subunit of CK-MB. Therefore only CK-B activity is measured, which is half of the CK-MB activity.

KIT COMPONENTS

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

Reagent A: 12 x 8 ml (liquid) blue cap

Reagent B: 2 x 12 ml (liquid) red cap

Composition in the test: imidazole buffer 100 mM pH 6.70, creatine phosphate 30 mM, glucose 20 mM, N-acetyl-L-cysteine 20 mM, magnesium acetate 10 mM, EDTA 2 mM, ADP 2 mM, NADP 2 mM, AMP 5 µM, Di(adenosine-5')pentaphosphate 10 µM, glucose-6-phosphate-dehydrogenase ≥ 1.5 kU/l, hexokinase ≥ 2.5 kU/l, Anti-CK-M polyclonal antibodies - inhibiting capacity > 2000 U/l.

Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

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

REAGENT PREPARATION

Serum as starter procedure:

Add 2 ml of reagent B to a vial of reagent A.

Stability of working reagent: 14 days at 2-8°C, away from light sources.

Reagent as starter procedure:

use separate reagents ready to use.

Stability: up to expiration date on labels at 2-8°C;

Stability since first opening of vials: ≥ 60 days at 2-8°C.

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" (GLP) guidelines.

SPECIMEN

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 serum specimen 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 enzymes and intermediates liberated from the erythrocytes may affect the lag phase and the side reactions may occur in the assay system.

TEST PROCEDURE (sample as starter)

Wavelength:	340 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette working reagent:	1 ml
preincubate at 37°C for 5 minutes.	
add sample:	40 µl
Mix, execute a first reading of absorbance after 1 minute, incubating at 37°C. Perform other 5 readings at 60 seconds intervals. Calculate the $\Delta A/\text{min}$.	

TEST PROCEDURE (reagent as starter)

Wavelength:	340 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent A:	1 ml
add sample:	50 µl
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent B:	250 µl
Mix, execute a first reading of absorbance after 1 minute, incubating at 37°C. Perform other 5 readings at 60 seconds intervals. Calculate the $\Delta A/\text{min}$.	

RESULTS CALCULATION

Perform calculation in units per litre, multiplying the $\Delta A/\text{min}$ by the factor as it is indicated.

Calculation in U/l: $\Delta A/\text{min} \times 8254$

Activity in µkat/l: $U/l \times 0.0167 = \mu\text{kat/l}$

EXPECTED VALUES

Serum: < 24 U/l (< 0.39 µkat/l)

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

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following control serum is available:

MB C008 CH CK-MB CONTROL 4 x 3 ml
with normal or close to normal control values

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity

the method is linear up to 1000 U/l.

If a $\Delta A/\text{min}$ of 0.250 is exceeded, it is suggested to dilute sample 1+9 with saline solution and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)

the limit of detection is 2 U/l.

Interferences

no interference was observed by the presence of:

lipids ≤ 2000 mg/dl

bilirubin ≤ 40 mg/dl

Hemoglobin interferences are possible.

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

Methods comparison

a comparison between Chema CK-MB FL and a commercially available product gave the following results:

$$\begin{aligned} \text{CK MB Chema} &= y \\ \text{CK MB competitor} &= x \\ n &= 75 \end{aligned}$$

$$y = 0.97x + 3.78 \text{ U/l} \quad r = 0.998$$

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

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.

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SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions