CK-MB FL IFCC/DGKC

MB F060 CH	6 x 10 ml
MB F120 CH	12 x 10 ml

INTENDED USE

Reagent for quantitative in vitro determination of creatine kinase MB in biological fluids.

SUMMARY OF TEST

Creatine kinase (CK) is an enzyme which is contained in heart, brain and skeletal muscles. 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 completely CK-MM activity (main part of the total CK activity) and 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.

CK-MB R1 **F060: 6 x 8 ml (liquid) blue cap**
F120: 12 x 8 ml (liquid) blue cap

CK-MB R2 **F060: 1 x 12 ml (liquid) red cap**
F120: 2 x 12 ml (liquid) red cap

Composition in the test: Buffer 100 mM pH 6.70, creatine phosphate 35 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 mM, Di(adenosine-5')pentaphosphate 10 μM, glucose-6-phosphate-dehydrogenase ≥ 1.5 kU/l, hexokinase ≥ 2.5 kU/l, Anti-CK-M monoclonal 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 R2 to a vial of reagent R1.

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: use preferably within 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)

Wavelenght:	340 nm
Ligthpath:	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 ΔA/min.

TEST PROCEDURE (reagent as starter)

Wavelenght:	340 nm
Ligthpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent R1:	1 ml
add sample:	50 μl
incubate at 37°C for 5 minutes.	
dispense in cuvette reagent R2:	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 ΔA/min.

RESULTS CALCULATION

Perform calculation in units per litre, multiplying the ΔA/min by the factor as it is indicated.

Calculation in U/l: ΔA/min x 8254

Activity in μkat/l: U/l x 0.0167 = μkat/l

EXPECTED VALUES

Serum: < 24 U/l (< 0.40 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:
QUANTINORM CHEMA
If required, a multiparametric, human based calibrator is available:
AUTOCAL H

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity
The method is linear up to 2000 U/l.
If a ΔA/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 4 U/l.

Interferences
no interference was observed by the presence of:

lipids	≤ 1700 mg/dl
bilirubin	≤ 46 mg/dl
hemoglobin	≤ 40 mg/dl
ascorbic acid	≤ 47 mg/dl

Precision

intra-assay (n=10)	mean (U/l)	SD (U/l)	CV%
sample 1	46.21	1.01	2.18
sample 2	101.46	1.80	1.77

inter-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	46.35	1.31	2.82
sample 2	101.64	1.03	1.01

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

CK MB Chema = y
CK MB competitor = x
n = 82

$y = 1.00 x + 0.46$ U/l $r^2 = 0.999$

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

HU Bergmeyer - Methods of enzymatic analysis, Vol. III (1987).
Clin. Chem. Lab. Med. 2002, 40(6), 635 - 642.

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SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use by date
	caution
	consult instructions for use

CE