CK-MB

IMMUNOINHIBITION

Intended use:

Immunoinhibition assay for the quantitative in vitro determination of the MB isoenzyme of creatine kinase in human serum and plasma.

Summary:

Creatine kinase (CK) is a dimeric enzyme occurring in four different forms: a mitochondrial isoenzyme and the cytosolic isoenzymes CK-MM (muscle type), CKBB (brain type) and CK-MB (myocardial type). The determination of CK-MB is an important element in the diagnosis of myocardial ischemia, e.g. in acute myocardial infarction or myocarditis. CK-MB is detectable in the blood about 3-8 hours after the onset of cardiac symptoms and can be detected over a lengthy period of time. CKMB may also appear in other clinical conditions such as rhabdomyolysis and stroke.

Within the scope of laboratory diagnostics, the determination of total CK, myoglobin and troponin T can contribute to the differentiation of these clinical pictures. The sensitivity of a CK-MB determination is dependent upon the time at which the sample was taken. Follow-up assays are therefore meaningful

Test principle:

Immunological UV assay

- Sample and addition of R1 (buffer/enzymes/coenzyme/antibody)
- Addition of R2 (buffer/substrate) and start of reaction.

Human CK-MB is composed of two subunits, CK-M and CK-B which both have an active site. With the aid of a polyclonal antibody to CK-M, the catalytic activity of CKM subunits in the sample is inhibited to 99.6% without affecting the CK-B subunits.The remaining CK-B activity, corresponding to half the CK-MB activity, is determined by the CK-MB method analogous to total CK. As the CK-BB isoenzyme only rarely appears in serum and the catalytic activity of the CK-M and CK-B subunits hardly differ, the catalytic activity of the CK-MB isoenzyme can be calculated from the measured CK-B activity by multiplying the result by

Reagent Concentration:

R1:	
Imidazole Buffer, pH 6.7	110 mmol/l
Glucose	21 mmol/l
Mg-Acetate	11 mmol/l
EDTA	2,1 mmol/l
NADP	2,4 mmol/l
N-Acetylcysteine	24 mmol/l
Hexokinase (HK)	2.5 U/l
PAK-CK-MM antibody (Sheep) Inhibitition capacity up to	2000U/l
Preservatives/Stabilizers	<1 %
R2:	
Tris Buffer, pH 9.1	50 mmol/l
ADP	2,4 mmol/l
AMP	6 mmol/l
Diadenosinpentaphosphate	12 µmol/l
G–6–P–DH	1.7 U/l
Creatinphosphate	186 mmol/l

Preparation and stability:

Serum start:

Working reagent is prepared by mixing gently 4 volumes of R1 with 1 volume of R2. DO NOT SHAKE!

This working solution is stable up to

3 days at +20°C to +25°C.
or 15 days at +2°C to +8°C

Unopened kid components are stable up to the expiry date at +2°C to +8°C

Substrate start:

R1: Ready for use
R2: Ready for use
Onboard stability: R1 28 days
R2 28 days

Specimen:

Centrifuge samples containing precipitate before performing the assay.
Collect serum using standard sampling tubes.
Heparinized- or EDTA-plasma
Stability: 2 days at +20°C to +25°C
7 days at +4°C to +8°C
4 weeks at -20°C

- Prior to the CK-MB assay, the total CK activity should be determined by the CKNAc method. The antibody is capable of inhibiting up to 2000 U/l CK-M subunit (37°C). Accordingly, CK-MM activities up to 1000 U/l (37°C) are completely inhibited. Therefore, samples with total CK activities above 1000 U/l (37°C) require dilution because complete inhibition is no longer assured.

Limitation - interference:

Criterion: Recovery within ± 10% of initial values.
Hemolysis: No significant interference up to an Index H of 600 (approximate hemoglobin concentration: 600 mg/dl).Icterus: No significant interference up to an index I of 80 for bilirubin (approximate bilirubin concentration: 80 mg/dl).
Lipemia (Intralipid): no significant interference up to an index L of 900 (approximate triglycerides concentration: 1800 mg/dl). There is poor correlation between turbidity and triglycerides concentration.
In patients with a disposition to macro-CK formation, implausibly high CK-MB values may be measured in relation to the total CK, since the macroforms mainly consist of CK-B subunits. As these patients have generally not suffered a myocardial infarction, additional diagnostic measures are necessary.

Measuring /reportable range:

7–600 U/l (0.08–16.67 µkat/l)
Determine samples with higher activities via the rerun function. On instruments without rerun function, manually dilute these samples with 0.9% NaCl or distilled/deionized water (e.g. 1:2). Multiply the result by the appropriate dilution factor (e.g. 3).

Testing procedure:

Applications for automated systems are available on request.

Materials provided

- Working solutions as described above

Additional materials required

- Calibrators and controls as indicated below
- 0.9% NaCl

Manual Testing Procedure: with Reagent start	
Wavelength:	340 nm,
Reaction temperature:	+37°C
Cuvette:	1 cm light path
Zero adjustment	air or distilled water
Cuvette	Normal
R1	800 µl
sample	40µl
Mix and incubate for 2 min. at 37°C. Then add:	
R2	200µl
Mix and incubate for 3 minutes. Read the absorbance A1. Perform other 4 readings at 60 seconds intervals.	
Calculation: ΔA/min x factor = U/L CK-MB in sample. Factor for 340 nm = 6300.	

Expected values:

On the basis of the optimized Standard IFCC method.
Myocardial infarction: There is high probability of myocardial damage when the following three conditions are fulfilled

		25°C	30°C	37°C
1	Ck MB	>10 U/l	>15 U/l	>24 U/l
2	The CK-MB-activity accounts for 6-25% of the total CK activity.			

CK varies with physical activity level and individual physiology in healthy individuals. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the CK results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings

Analytical sensitivity (lower detection limit):

Normal cuvette: 3 U/l
The lower detection limit represents the lowest measurable CK concentration that can be distinguished from zero.

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Method comparison:

A comparison of the BIOANALYTIC CK-MB (y) with a commercial obtainable assay (x) gave the following result:

y = 1.074 x - 3.01; r = 0.999

Imprecision:

Reproducibility was determined using controls between day (n = 20). The followingresults were obtained

Repeatibility / Within run			
	Mean	CV	N
Sample 1	40,5 U/L	3,7 %	20
Sample 2	81,6 U/L	3,4 %	20
Reproducibility / Betwen day			
	Mean	CV	N
Sample 1	40,5	5,1	25
Sample 2	81,6	3,4	25

Quality Control:

Control Serum

BIOANALYTIC CK, CK-MB CONTROL	3 x 1 ml	# B10821
BIOCON N	5 x 5 ml	# B10814
BIOCON P	5 x 5 ml	# B10817

For quality control, use a suitable control material. The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:

S1: %9 NaCl

S2: BIOCAL H 5 x 3 ml #B11895

Calibration stability

It is suggested to use Calibrator products produced by Bioanaliytic. It is suggested to use supplementary calibrator (pure water or 0.9% NaCl) to conduct 2-point calibration. The calibration curve is formed automatically. When lot number is changed or QC is invalid, calibration shall be conducted again. Recalibrate the assay every 30 days under ideal conditions, or when the following occur:

Change in reagent lot or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced(Halogen Lamp)

Literature:

1. Adams JE, Abendschein DR, Jaffe AS. Biochemical markers of myocardial injury: Is MB Creatine kinase the choice for the 1990? Circulation 1993;88:750-763.
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4. Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes Preanalytica Variables, Broschüre in Samples: From The Patient to the Laboratory. Darmstadt: GIT Verlag 1996
5. Jockers-Wretou E, Pileider G. Clin Chim Acta 1975;58:223.
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9. Stein W. Med. Welt 1985;36:572
10. Szasz G Busch EW. Third European Congress of Clinical Chemistry Brighton, England 3.-8. Juni 1979 (Abstract)
11. Würzburg U et al. Klin Wschr 1976;54:357

Order information (Cat No.) :

CC365	CR365	B24090	B28090	B32091	B36091
CC365	BCKMB250	B24091	B28091	B33090	B37090
OL365	BCKMB125	B25090	B28092	B33091	B37091
OL366	BCKMB50	B25091	B30090	B33092	B80090
KL365	B21090	B25092	B30091	B34090	B80091
KL366	B21091	B27090	B30092	B34091	B80092
AB365	B21092	B27091	B31090	B35090	B80093
AB366	B22090	B27092	B32090	B36090	

Manufacturer

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SYMBOLS

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

ISO 9001:2015
ISO 13485:2016