CK-MB DS

unitest

MONOCLONAL

Double Sensitivity method for CK-MB determination in serum or plasma

SUMMARY

Creatine Kinase (CK) is a dimeric enzyme occuring in three citoplasmatic forms. Isoenzymes CK-MM is a muscle enzyme, CK-BB is a brain enzyme and CK-MB is the heart enzyme.
Serum increase levels of total CK and isoenzyme CK-MB are indicators of myocardial injury. After an acute myocardial infarction, in approximately 55% of the cases, the highest increase of CK and CK-MB is simultaneously produced, while in 45% of the cases the highest increase of CK-MB procedes the one of total CK.

PRINCIPLE

The method is based on the specific inhibition of the CK-M subunit with monoclonal antibodies anti-CK-M. The antibodies inhibit not only the MM isoenzyme but also the M subunit corresponding to CK-MB. The reaction system is the following:

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{D-Glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{D-Glucose-6-phosphate}$$

$$\text{D-glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G-6-PDH}} \text{6-phospho-D-gluconolactone} + \text{NADPH} + \text{H}^+$$

$$\text{6-phospho-D-gluconolactone} + \text{H}_2\text{O} \xrightarrow{\text{6-PGL}} \text{6-phospho-D-gluconate}$$

$$\text{6-phospho-D-gluconate} + \text{NADP}^+ \xrightarrow{\text{6-PGDH}} \text{D-ribulose-5-phosphate} + \text{CO}_2 + \text{NADPH}$$

The use of 6-phospho-D-gluconolactonase (6-PGL) and 6-phosphogluconate dehydrogenase (6-PGDH) increases sensitivity by releasing another molecule of NADPH into the reaction, duplicating the signal.
As the CK-BB isoenzyme appears only rarely in serum and the catalytic activity of the CK-M and the CK-B subunits hardly differ, the catalytic activity of the CK-MB isoenzyme can be calculated measuring CK-B activity by multiplying the result by 2.

HK: hexoquinase; G-6-PDH: glucose-6-phosphate dehydrogenase; 6-PGL: 6-phospho-D-gluconolactonase; 6-PGDH: 6-phosphogluconate dehydrogenase; ATP/ADP: adenosine 5' tri/diphosphate; NADH: nicotinamide-adenine dinucleotide (reduced); NAD^+: nicotinamide-adenine dinucleotide.

PROVIDED REAGENTS

A. Reagent A: solution with creatine phosphate, anti-CK-M antibodies and reactive components in sufficient quantities to obtain the following final concentrations:

Creatine phosphate	30 mmol/l
ADP	2 mmol/l
Glucose	20 mmol/l
NADP	2 mmol/l
Hexokinase (HK)	≥ 2 500 U/l
Glucose-6-P-dehydrogenase (G-6-PDH)	≥ 2 000 U/l
Magnesium acetate	10 mmol/l
AMP	5 mmol/l
Di (adenosine-5´) pentaphosphate	10 umol/l
N-acetyl-cysteine (NAC)	20 mmol/l
6-phospho-gluconolactonase (6-PGL)	≥ 200 U/l
6-P-gluconate dehydrogenase (6-PGDH)	≥ 400 U/l

Monoclonal antibodies capable of inhibiting 1000 U/l of CK-M (at 25°C) or 2000 U/l of CK-M (at 37°C).
B. Reagent B: 100 mmol/l imidazole buffer solution, pH 6.7.
Control: vial containing lyophilized human CK-MB (see attached table for theoretic value).

INSTRUCTIONS FOR USE

Reagent A: add 2.5 ml Reagent B into a Reagent A vial. Cap and shake until complete dissolution.
Reagent B: ready to use.
Control: open the vial carefully trying to not spill the content. Reconstitute with the distilled water volume stated in the label. Cap and wait for 5 minutes. Dissolve the content of the vial completely by inversion. The reconstituted CK-MB Control is treated in the same way as an unknown sample.

WARNINGS

Reagents are for “in vitro” diagnostic use. Buffer contains azide.
Do not ingest. Avoid contact with skin and eyes. If spilt or splash, wash affected area with plenty of water.
The Control has been tested for HIV, HCV and HBV being found non-reactive. Nonetheless, it should be handled as infectious material.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable at 2-10°C until the expiration date stated on the box.
Reconstituted Reagent A: stable at 2-10°C for up to 7 days after reconstitution date.

Reconstituted Control: stable at 2-10°C for up to 3 days or for up to 3 months at -20°C. Do not freeze and thaw repeatedly.

INSTABILITY OR DETERIORATION OF REAGENTS
Failure to recover control values within the assigned range could indicate deterioration and the reagents should not be used.
When the spectrophotometer has been set to zero with distilled water, absorbance readings of the reconstituted Reagent A higher than 0.800 O.D. (at 340 nm) indicate its deterioration.

SAMPLE
Serum or plasma
a) Collection: obtain in the usual way.
b) Additives: when using plasma, heparin or EDTA must be used as anticoagulant. The use of Wiener lab. **Anticoagulante W** Wiener lab is recommended.
c) Known interfering substances: sera with visible or intense hemolysis should not be used as they produce false increased values.
No interferences are observed with hemoglobin up to 50 mg/dl, bilirubin up to 2,5 mg/dl (25 mg/l) and heparin up to 20 U/ml. See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: sample should be fresh. In case it cannot be processed immediately, the sample may be stored for up to 12 hours at room temperature (< 25°C), for up to 3 days at 2-10°C, or for up to 30 days at -20°C.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer.
- Micropipettes and pipettes for measuring the stated volumes
- Water bath at the temperature indicated under PROCEDURE.
- Stopwatch.

ASSAY CONDITIONS
(Increase of Absorbance)
- Wavelength: 340 nm (Hg 334 or 366).
- Reaction temperature: 25, 30 or 37°C.
- Reaction time: 10 minutes
- Sample and reagent volumes: may vary proportionally (e.g. 40 ul sample + 1 ml reconstituted Reagent A or 20 ul sample + 500 ul reconstituted Reagent A).

PROCEDURE
Set the instrument to zero O.D. with distilled water. See INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS. In a cuvette at the selected temperature (25, 30 or 37°C) place:

Reconstituted Reagent A	2.5 ml
Pre-incubate a few minutes. Then add:	
Sample	100 ul

Mix immediately by inversion. Wait 5 minutes. Adjust absorbance to a reference reading and simultaneously start stopwatch. Measure absorbance every minute during 5 minutes. Determine average change in Absorbance/min (ΔA/min), substracting each reading from the previous one and averaging these values. Use this mean for calculations.

CALCULATIONS
CK MB U/l = ΔA/min x factor
Measure at 340 nm: CK-MB (U/l) = ΔA/min x 4,127
Measure at Hg 334: CK-MB (U/l) = ΔA/min x 4,207
Measure at Hg 366: CK-MB (U/l) = ΔA/min x 7,429

The calculation factors above mentioned, already include the correction needed to convert the value of CK-B into CK-MB.

QUALITY CONTROL METHOD
Each time the test is run, analyze two levels of a quality control material (**CK-MB Control**) with known CK-MB activity.

REFERENCE VALUES

Temperature	25°C[1]	30°C[2]	37°C[2]
Values	10 U/l	16 U/l	25 U/l

[1] Stein, W. - Med. Welt. 36:572, 1985.
[2] Calculated values

It is recommended that each laboratory establishes its own reference values.

INTERPRETATION OF RESULTS
A high probability of myocardial damage exists if the following conditions are simultaneously met:
1- Total CK activity exceeds the following normal ranges:

Temperature	25°C[1]	30°C[2]	37°C[3]
Men	10-80 U/l	15-130 U/l	24-195 U/l
Women	10-70 U/l	15-110 U/l	24-170 U/l

[1] Stein W. - Med. Welt. 1985; 36:572.
[2] Szaz G., Busch E.W. - Third European Congress of Clinical Chemistry, Brighton, England, 3-8 June 1979.
[3] Calculated

2- CK-MB activity exceeds normal values. See REFERENCE VALUES.
3- The CK-MB percentage in found between the 6-20% of the total CK value.
If the percentage is bellow 6% there is probably damage to the skeletal muscle. If the percentage is over 20% of the total CK value the presence of a macro kind of CK (atypical CK) which is not inhibited by the anti CK-M antibodies, can be suspected.
The atypical CK presence may be determined by:
a) Isoenzyme persistence for more than 48 hours (the CK-MB decays approximately at 30-48 hours after the onset of the infarction).
b) Stability when treating the sample at 40°C during 20 minutes.
c) Electrophoretic analysis (a band between MM and MB isoenzymes is obtained).

The possibility of recent infarction exists if myocardial damage is suspected and values are below the normal range. In this case repeat the assay after 4 hours.

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
Samples with total CK activity over 1000 U/l (at 25ºC) or over 2000 U/l (at 37ºC), should be diluted with saline solution (0.9% sodium chloride). The obtained result should be multiplied by the dilution performed.

PERFORMANCE

The assays were performed in Express plus analyzer(*). If the kit is performed using manual procedure, user must validate that similar performed to that stated below is obtained.
a) Reproducibility: processing according to EP5A protocol of NCCLS (National Committee on Clinical Laboratory Standards), the following values were obtained:

Within-run precision

Level	S.D.	C.V.
39.5 U/l	± 0.6 U/l	1.44 %
183.4 U/l	± 1.9 U/l	1.05 %

Total-run precision

Level	S.D.	C.V.
39.5 U/l	± 0.8 U/l	1.90 %
183.4 U/l	± 2.6 U/l	1.39 %

b) Analytical sensitivity: 7.1 U/l.
c) Linearity: reaction is linear up to 350 U/l. For higher values, dilute the sample with saline solution, repeat the determination and multiply the obtained result according to the dilution factor.
d) Correlation: CK-MB values of 84 specimens were determined using **CK-MB DS UV unitest** and a commercial kit of the same methodology. Correlation coefficient with serum and plasma samples was:
r = 0.9985, slope b = 0.9779 and intercept was a = -1.5464.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user's manual of the autoanalyzer in use.

WIENER LAB. PROVIDES

- 28 vials x 2.5 ml + 2 Control Sera c.s. 1 ml (Cat. Nº 1271354).
- 28 vials x 2.5 ml + 2 Control Sera c.s. 1 ml (Cat. Nº 1009323).

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SYMBOLS

The following symbols are used in packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
Σ	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
⟶	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
Xn	Harmful
	Corrosive / Caustic
Xi	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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