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CREATINE KINASE MB (CK-MB) CE

STABLE LIQUID REAGENT

Cat. No.:	48861	48863
	125 ml	10x25 ml
	(1x100 ml+1x25ml)	(10x20 ml+ 10x5 ml)

Reagent kit for the determination of creatine kinase-MB activity based upon DGKC and IFCC recommendations.

Principle

This procedure involves measurement of CK activity in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity, of CK-MM and half of the activity of CK-MB while not affecting the B subunit activity of CK-MB and CK-BB. Then we use the CK method to quantitatively determine CK-B activity. The CK-MB activity is obtained by multiplying the CK-B activity by two. The sample is incubated in the CK-MB reagent which includes the anti-CK-M antibody. The activity of the noninhibited CK-B is then determined using the following series of reaction:

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

$$\textit{ATP} + \textit{glucose} \xrightarrow{\text{HK}} \textit{Glucose-6-Phosphate} + \textit{ADP}$$

$$\textit{Glucose-6-Phosphate} + \textit{NADP}^{+} \xrightarrow{\text{G6-PDH}} \textit{Gluconate-6-Phosphate} + \textit{NADPH} + \textit{H}^{+}$$

G-6-PDH=Glucose-6-Phosphate Dehydrogenase, CK=Creatine kinase, HK=Hexokinase

Reference values

CK-MB activity: **0-24 U/l (37°C) (0-0,4 µkat/l)**
CK-MB% < 5%

It is recommended that each laboratory should assign its own normal range. 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 macro forms mainly consist of CK-B subunits. As these patients have generally not suffered a myocardial infarction, additional diagnostic measures are necessary.

Reagents

1. Reagent (R1)

Anti-human polyclonal CK/M antibody (Goat) sufficient to inhibit up to 2000 U/l of subunit at 37°C.

2.Reagent (R2)

Imidazole buffer pH: 6.70	100 mmol/l
Magnesium acetate	10 mmol/l
N-acetylcysteine	20 mmol/l
ADP	2 mmol/l
AMP	5 mmol/l
NADP	2 mmol/l
D-Glucose	20 mmol/l
Diadenosine pentaphosphate	10 µmol/l
EDTA	2 mmol/l
Hexokinase	≥3500 U/l
Glucose-6-phosphate dehydrogenase	2000 U/l
Creatine-phosphate	30 mmol/l

Precaution

Reagents contain sodium azide (0.1%) as preservative. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent.

Sample

Serum free of haemolysis. Storage: 1 week at 2-8°C or frozen (-20°C).

PROCEDURE

Preparation of working reagent

• One-reagent procedure:

Mix 4 volumes of reagent 1 with 1 volume of reagent 2.

Stability: at 20-25 °C : 5 days
at 2-8 °C: 2 weeks

• Two-reagent procedure: reagents are ready for use.

If the absorbance of working reagent is higher than 1.0 at 334 nm the reagent can not be used.

Assay conditions

Wavelength:	334-340 nm
Temperature:	37 °C
Cuvette:	1 cm light path
Read against:	distilled water
Method:	kinetic (increasing)

• **One-reagent procedure**

Working reagent	1 ml
Sample or control	50 µl

Mix and after a 5-minute incubation, measure the change of absorbance per minute (ΔA/min) during 3 minutes.

• **Two-reagent procedure**

R1	800 µl
Sample	50 µl

Mix, incubate for one minute at 37 °C and add:

R2	200 µl

Mix and after a 5-minute incubation, measure the change of absorbance per minute (ΔA/min) during 2 minutes.

Calibration (37°C, DGKC and IFCC method)

S1: Distilled water
S2: Randox CK-MB calibrator

Calibration frequency

Two point calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} x C_{standard} = C_{sample}$$

A = Absorbance, C = Concentration

Calculation using factor

1.CK-B activity (U/l)=ΔA/min x 3500; =(µkat/l) =ΔA/min x58,3
2.CK-MB activity (U/l)=CK-B × 2

3.CK-MB%= $\frac{CK-MB\ activity}{total\ CK\ activity} \times 100$

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 400 analyzer (37°C).

Linearity

If the total CK activity is higher than 1200 U/l (20µkat/l) dilute the sample in ratio of 1:10 with physiological saline solution before assay of CK-MB.

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 6.00 U/l (0,1µkat/l) CK-MB activity at 334 nm.

Precision

	Repeatability				
	Average activity (U/l)	One-reagent procedure		Two-reagent procedure	
		SD	CV%	SD	CV%
Serum I.	22	0.36	1.64	0.37	1.70
Serum II.	219	4.75	2.17	4.20	1.92

	Reproducibility		
	Average activity (U/l)	One reagent procedure SD	Two reagent procedure CV%
Serum I.	110.5	1.87	1.69
Serum II.	211.3	7.76	3.67

Correlation

A comparative study has been performed between our reagent and another commercial CK-MB liquid reagent on 37 human samples.
The parameters of linear regression are as follows:
Correlation coefficient: r= 0.9990
Linear regression: y (U/l)= 0.951x + 2.140
(x= other commercial reagent, y= own reagent).

Selectivity

Haemolysis, lipaemic and icteric serum interfere with the test.

NOTE

The method will also measure any CK-BB isoenzyme present in serum. The activity of this isoenzyme is usually negligible, however, if a significant amount of CK-BB activity is present the CK-MB activity will be overestimated.

A macro form of BB (immunoglobulin complexed) has been observed which will be measured as a B in this assay. If the measured CK-B activity is greater than 20% of total CK activity the presence of macro BB should be suspected.
Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography

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2.Chan D.W., Taylor E., Frye R. és Blitzer R.L., Clin.Chem., 31, (1985) 465
3.Morin L., Clin. Chem., 23/4,(1977), 646