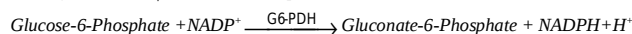
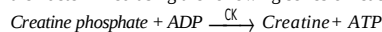


Reagent kit for the determination of creatine kinase-MB activity based upon DGKC and IFCC recommendations.

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:



G-6-PDH=Glucose-6-Phosphate Dehydrogenase

CK=Creatine kinase, HK=Hexokinase

Reference values

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

Precautions

The reagent 1 contains 0.1 % sodium azide. 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

Dissolve the reagent 2 (R2) in buffer (R1).

Stability:	at 20-25°C:	24 hours
	at 2-8°C:	3 days

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)

Pipette into cuvette

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.

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

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}$$

A = Absorbance
C = Concentration

Calculation using factor

1. CK-B activity (U/l)=ΔA/min x 3750; (µkat/l) = ΔA/min x 62,5

2. CK-MB activity (U/l)=CK-B × 2

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

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 600 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 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 7.50 U/l (0,125 µkat/l) CK-MB activity at 334 nm.

Precision

	Reproducibility		
	Average activity (U/l)	SD	CV%
sample I	48.4	2.15	4.44
sample II	94.7	1.60	1.69

	Repeatability		
	Average activity (U/l)	SD	CV%
sample I	14.3	0.44	3.12
sample II	249	2.50	1.01

Correlation

Comparative studies were done to compare our reagent with another commercial CK-MB reagent. The results from these studies are detailed below.

Correlation coefficient: r=0.9968

Linear regression: y (U/l)= 0.995x+1.138

(x= other commercial reagent, y= own reagent).

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

-  For in vitro diagnostic use
-  Use by (last day of the month)
-  Temperature limitation
-  Batch Code
-  Code

Bibliography

1. Morisok I. M., Clayton J. és Fine J.S. Clin. Chem., 34, (1988), 535
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