CREATINE KINASE MB LS 4+1

IVD

Quantitative determination of Creatine Kinase - MB in Serum or Plasma. Immuno inhibition, Kinetic UV. Optimized DGKC / IFCC

REF CC1160 R1: 3x20 mL + R2: 1x15 mL **REF CC1162** R1:3x40 mL + R2: 1x30 mL

METHOD AND PRINCIPLE

Optimized UV test according to DGKC (German Society of Clinical Chemistry) and IFCC (International Federation of Clinical Chemistry) for CK with inhibition of CK-M isoenzymes by monoclonal antibodies. CK-MB consists of the subunits CK-M and CK-B. Specific antibodies against CK-M inhibit the complete CK-MM activity (main part of the total CK activity) and the CK-M-subunit of CK-MB. Only CK-B activity is measured, which is half of the CK-MB.

$$\text{Creatine Phosphate + ADP} \xrightarrow{\text{CK-B}} \text{Creatine + ATP}$$

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

$$\text{G-6-P + NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{D-Gluconate-6-phosphate + NADPH + H}^{+}$$

Increasing the concentration of NADPH, causes an increase of absorbance of solution, measurable fotometrically at 340 nm, proportionally to activity of enzyme in the sample.

CLINICAL SIGNIFICANCE

The major medical application of CK-MB assays is in adults for the diagnosis of acute myocardial infarction (AMI) or, the differentiation of myocardial injury from skeletal muscle damage. Creatine Kinase MB is considered one of the best laboratory indicators of AMI. CK-MB determination within the proper time frame after infarction is most critical, the useful interval ("window") being from about 10 to 24 hour after the infarction. Its detection is of importance in determining the degree of the injury and the efficacy of the treatment.

REAGENT COMPOSITION

Reagent (R1)

Imidazole buffer, 6.7	100 mmol/L
Glucose	20 mmol/L
N-Acetylcysteine (NAC)	20 mmol/L
Magnesium acetate	10 mmol/L
NADP	2.5 mmol/L
Hexokinase (HK)	≥ 4 KU/L
EDTA-Na2	2 mmol/L
Monoclonal antibodies against human CK-M	2500 U/L

Reagent (R2)

Creatine phosphate	30 mmol/L
AMP	5 mmol/L
ADP	2 mmol/L
di-adenosine-pentaphosphate	10 µmol/L
G-6P-DH	≥ 1.5 KU/L

REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera. After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid bacterial contamination, degradation from direct light and evaporation.

For the Sample Starter Procedure, prepare a Work Solution by mixing 4 parts of R1 and 1 part of R2 (E.g. 20 mL of R1 + 5 mL of R2). Stability: Stability: 24 hours at 15-25 ° C, 15 days at 2-8 ° C.

For the Substrate Starter Procedure, reagents R1 and R2 are ready to use and stable until the expiry date if stored at the temperature shown on the label and avoid contamination, prolonged exposure to direct light and evaporation.

SPECIMEN

Serum, heparin plasma or EDTA plasma.
Stability: 2 days at 20° - 25°C; 7 days at 4°- 8°C; 4weeks at -20 °C.
Only freeze once. Discard contaminated specimens.

PROCEDURE

Wavelength:	340 nm
Temperature:	37°C
Measurement:	against distilled water

Substrate Start procedure:

Reagent (R1)	800 µL
Sample / Calibrator	40 µL

Mix and after 3 minutes add:

Reagent (R2)	200 µL

Mix, read Absorbance (Abs 1) after 2 minutes and start stopwatch. Read Absorbance again after 1, 2, 3, 4 and 5 minutes.
Calculate ΔAbs/min (average)

Sample Start procedure:

Working Reagent	1000 µL
Sample	40 µL

Mix, read Absorbance (Abs 1) after 5 minutes and start stopwatch. Read Absorbance again after 1, 2, 3, 4 and 5 minutes.
Calculate ΔAbs/min (average).

CALCULATION

Calculation Factor (reading at 340 nm in 1 cm cuvette):
CK-MB (U/L) = ΔAbs/min (average) x 8255

Multiparametric Calibrator:
Calculate a specific factor using a certificate multiparametric calibrator.

$$\text{Factor} = \frac{\text{Calibrator Concentration}}{\Delta\text{Abs/min (average).}}$$

CK-MB (U/L) = ΔAbs/min (average) x Factor

Conversion Factor: CK-MB [U/L] x 0.0167 = CK [µkat/L] = CK[µmol/sec/L]

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CALIBRATION

The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.

Use **MTD Diagnostics Calibrator**

Chemistry Multicalibrator - REF CAL1010 (10x3 mL)

QUALITY CONTROL

Normal and abnormal control sera of known enzymatic activity should be analysed routinely with each group of unknown samples utilizing **MTD Diagnostics** Quality Control Material:

Chemistry Control N - REF CNN1010 10x5 mL (Level 1)
Chemistry Control P - REF CNP1020 10x5 mL (Level 2)

EXPECTED VALUES

Myocardial infarction: the risk of myocardial infarction is high if following three conditions are fulfilled:

- ✓ CK (Men) > 190 U/L
- ✓ CK (Women) > 167 U/L
- ✓ CK-MB > 24 U/L
- ✓ CK-MB activity is between 6 and 25 % of total CK activity.

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:
Low Level: Samples= 20; Average = 26; S.D. = 0.7; CV = 2.61%
High Level: Samples = 20; Average = 106; S.D. = 1,03; CV = 0.97%

ACCURACY (CORRELATION): A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

y=1.00x + 2.08 ; r=1.000

SENSITIVITY: 2 U/L

LINEARITY: 2 – 200 U/L

SPECIFICITY / INTERFERENCES

No interference was observed by ascorbic acid up to 30 mg/dL, conjugated and unconjugated bilirubin up to 25 mg/dL and lipemia up to 900 mg/dL triglycerides. Haemoglobin interferes even in minimum concentrations as from 25 mg/dL. For further information on interfering substances refer to Young DS.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact your distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

PRECAUTIONS

The product does not contain any other hazardous substances or mixtures according to EC Regulation No. 1272/2008 (CLP) or their concentrations are such that they are not considered to be persistent, bioaccumulative or toxic (PBT). Therefore, it is not subject to the special labeling required by the aforementioned regulation. The product is labeled according to the directive for CE marking (98/79 / EC). Sodium Azide less than 0.1%.
However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, apply the following first aid:
S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.

FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

SIMBOLOGY

CE	CE Mark (EC Directive 98/79)		
IVD	In Vitro Diagnostic		Temperature Limitation
	Consult instructions for use		Contains sufficient for <n> test
REF	Catalog Number		Use By
LOT	Batch Code		Manufacturer

BIBLIOGRAPHY

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