Creatine Kinase MB

CYPRESS DIAGNOSTICS

CE

IVD

Immuno-inhibition. UV. Kinetic
Liquid

Store at 2-8 °C

Configuration

REF	HBEL05
VOL	60 + 15 mL
Reagent 1	1 x 60 mL
Reagent 2	1 x 15 mL
CK (NAC & MB) Control	1 x Lyoph.-2 mL
Instrument	Universal

Intended use

Quantitative determination of creatine kinase MB in human serum or heparinized plasma.
For *in vitro* diagnostic use only. For professional use only.

Clinical significance

CK-MB is composed of two moieties CK-M (from muscles) and CK-B (from brain). CK-MB is usually present in serum at low concentration; it is increased after an acute infarct or myocardium and later descends at normal levels. Rarely, it can also be increased in skeletal muscle damage.[5,6]
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Principle

A specific antibody inhibits the CK-M moiety without affecting the CK-B moiety. The CK-B fraction accounts for one half of the activity of CK-MB, it is determined by the NAC-activated method. The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of CK-B present in the samples.

Reagent composition

Reagent 1	Imidazole pH 6,7 (125 mmol/L) D-Glucose (25 mmol/L) N-acetylcysteine (25 mmol/L) Magnesium acetate (12,5 mmol/L) $NADP^+$ (2,52 mmol/L) EDTA (2,02 mmol/L) Hexokinase (HK) (≥ 6800 U/L)
Reagent 2	ADP (15,2 mmol/L) AMP (25 mmol/L) Diadenosine -5-P (103 mmol/L) G-6-PDH (≥ 8800 U/L) Creatine-phosphate (250 mmol/L) *Anti-CK-M (2000 U/L)
CK (NAC & MB) Control	Lyophilized human serum (2 mL) Value (assay at 37°C) indicated on label

*Anti human polyclonal CK-M antibody (sheep) is sufficient to inhibit up to 2000 U/L of CK-M moiety.

Precautions

- The control is prepared from human sera, which have been tested and are found to be non-reactive for HBsAG and antibodies to HCV and HIV. However, all human specimens should be considered potentially infectious.
- EUH210: Safety data sheet available on request.
- All body fluid samples should be considered potentially infectious materials and the appropriate precautions should be taken. Wear personal protective equipment such as gloves, safety glasses, lab coats or aprons when working with possible biohazard contaminants.
- Use Good Laboratory Practices (GLP) when handling this product.
- Please refer to the MSDS, available on our website, for further information.

Preparation

Working reagent: mix 4 volumes of R1 with 1 volume of R2. After mixing, allow to stand for 30 minutes prior to use. The stability of the working reagent is 7 days at 2-8 °C or 12 hours at room temperature (15-25 °C).
Control: dissolve the contents in 2 mL of distilled water. Cap vial and mix gently to dissolve the contents. Stability: 8 hours at 15-25 °C, 5 days at 2-8 °C or 1 month at -20 °C. Bring at room temperature for about 30 min before use.

Storage, stability and disposal

All the components of the kit are stable up to the date of expiration as specified on the label, when stored tightly closed, protected from light and contaminations prevented during their use. Storage temperature for this kit is 2-8 °C.
The reagent should be a clear solution. If turbidity or precipitation has occurred or if blank absorbance at 340 nm ≥ 1,20, the reagent should be discarded.
Do not use the product if deterioration or contamination is suspected or beyond the expiration date or open container stability period. Dispose unused or deteriorated product and waste in compliance with local regulations.

Additional material required but not provided

- Spectrophotometer or colorimeter measuring at 340 nm
- Thermostatic bath at 25 °C, 30 °C or 37 °C (± 0,1 °C).
- Matched cuvettes 1,0 cm light path
- General laboratory equipment

Samples

Serum free of hemolysis or heparin plasma: stability 7 days at 2-8 °C, protected from light.[1]
CK-MB activity decreases a 10% after 24 hours at 4 °C or 1 hour at 25 °C.

Procedure for Manual Method

Make sure the reagents and samples are at room temperature.
1. Wavelength 340 nm; Temperature 25, 30 , 37 °C; Cuvette (1 cm light path).
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

For Sample	40 µL Sample + 1 mL Working reagent
Mix and incubate for 10 min. Read initial absorbance (A1) of the sample, start the stopwatch and read again after 5 min (A2). Calculate the difference between the absorbances: ΔA = A2 - A1.	

Procedure for CYANStart/CYANSmart

Make sure the reagents and samples are at room temperature.
1. Wavelength 340 nm; Temperature 25, 30 , 37 °C; Cuvette (1 cm light path).
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

For Sample	40 µL Sample + 1 mL Working reagent
Mix and incubate for 10 minutes at room temperature. After the incubation time, aspirate and measure the sample.	

Calculation

Manual method:
ΔA x 825 = U/L CK-B ΔA x 1651 = U/L CK-MB

Kinetic method:
ΔA/min x 4125 = U/L CK-B ΔA/min x 8255 = U/L CK-MB

One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per liter of sample (U/L).

Conversion Factor: µkat/L = 0,0167 x U/L

Percentage of CK-MB activity in sample:
% CK-MB activity = (CK-MB activity / CK Total Activity) x 100

Temperature conversion factors

To correct results to other temperatures, multiply by:

Assay Temperature	Desired Temperature		
	25°C	30°C	37°C
25°C	1,00	1,53	2,38
30°C	0,65	1,00	1,56
37°C	0,42	0,64	1,00

Quality control

Control sera are recommended to monitor the performance of assay procedures. Use the CK (NAC&MB) control (HBC08) included in the kit.
Prepare and measure these controls the same as samples. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference values

CK-MB activity - 25 °C	≤ 10 U/L (≤ 0,17 µkat/L)
CK-MB activity - 30 °C	≤ 15 U/L (≤ 0,25 µkat/L)
CK-MB activity - 37 °C	≤ 24 U/L (≤ 0,40 µkat/L)
CK total (CK-NAC) activity - Men - 25 °C	≤ 80 U/L (≤ 1,34 µkat/L)
CK total (CK-NAC) activity - Women -25 °C	≤ 70 U/L (≤ 1,17 µkat/L)
CK total (CK-NAC) activity - Men - 30 °C	≤ 130 U/L (≤ 2,17 µkat/L)
CK total (CK-NAC) activity - Women - 30 °C	≤ 110 U/L (≤ 1,84 µkat/L)
CK total (CK-NAC) activity - Men - 37 °C	≤ 195 U/L (≤ 3,26 µkat/L)
CK total (CK-NAC) activity - Women - 37 °C	≤ 170 U/L (≤ 2,84 µkat/L)

The heart infarct probability is high when all three following conditions are fulfilled:
1. CK-MB activity increases above the indicated values
2. CK-NAC activity increases above the indicated values
3. CK-MB activity is 6 - 25 % of total CK activity

These values are for orientation purpose. Each laboratory should establish its own reference range.

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be

Performance characteristics

Measuring range: **from 1,9 U/L (detection limit) to 318 U/L (linearity limit). If the obtained results are greater than 318 U/L, dilute the sample 1:2 with saline solution, repeat the determination, and multiply the result by factor 2.**

Precision:

	intra-assay (n=20)	
Mean (U/L)	33,7	166,5
SD	1,00	3,76
CV (%)	2,96	2,26

inter-assay (n=20)	
31,3	161
1,19	3,47
3,81	2,15

Sensitivity: **1 U/L = 0,000134 ΔA/min**

Accuracy: **Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.**
The results of the performance characteristics depend on the analyzer used.

Interferences

Bilirubin (mixed isomers): less than 10% interference up to 600 µmol/L bilirubin.
Hemolysis: less than 10% interference up to 1,25 g/L hemoglobin.
Lipemia: less than 10% interference up to 2,5 g/L intralipid.
A list of drugs and other interfering substances with CK-MB determination has been reported by Young et al.[3,4]

Limitations

- **The method will also measure any CK-BB isoenzyme present in serum. The activity of the isoenzyme is 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 B in the assay. If the measured CK-B activity exceeds 20% of the total CK activity, the presence of macro BB should be suspected.**

Notes

1. **For best use of this kit on a Cypress Diagnostics analyzer, we kindly advise to follow the application sheets of the respective analyzer. Please log in to our website (www.diagnostics.be) as a registered user to download the latest application sheets, which are located under the section of the corresponding analyzer. Compatible Cypress analyzers: CYANSmart, CYANStart, CYANExpert 130, CYANVision**

Bibliography

1. **Abbot B et al. Creatine Kinase. Kaplan A et al. Clin Chem The C.V. Mosby CO. St Louis. Toronto. Princeton 1984; 1112-1116**
2. **Gerhardt W et al. Clin Chem 1979 (25/7): 1274-1280**
3. **Young DS. Effects of drugs on Clinical Lab. Tests. 4th ed AACC Press 1995**
4. **Young DS. Effects of diseases on Clinical Lab. Tests, 4th ed AACC 2001**
5. **Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999**
6. **Tietz N W et al. Clinical Guide to Laboratory tests, 3rd ed AACC 1995**
7. **Mathieu M. et al. Ann. Biol. Clin. 1982 (40): 87**
8. **Neumeier D. et al. Clin Chim. Acta 1976 (73): 445**

Notice: Any serious incident that has occurred in relation to the device shall be reported to Cypress Diagnostics and the competent authority of the Member State in which the user and/or patient is established.

2021-12, Rev. 8.0

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be