

Diagnostic reagent for quantitative in vitro determination of creatine kinase (CK-MB) in human serum or plasma on photometric systems

TEST PARAMETERS

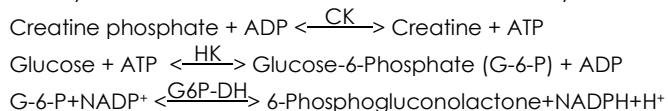
Method: UV, Kinetic, Increasing Reaction, opt. DGKC / IFCC
 Wavelength: 340 nm, Hg 334 nm
 Temperature: 37 °C
 Sample: Serum, plasma
 Linearity: up to 2000 U/L
 Sensitivity: The lower limit of detection is 2 U/L.

SUMMARY [1,2]

Creatine kinase (CK) is an enzyme which consists of isoenzymes mainly of the muscle (CK-M) and the brain (CK-B). CK exists in serum in dimeric form as CK-MM, CK-MB, CK-BB and as macroenzyme. Measurement of CK-MB is a specific test for detection of cardiac muscle damage and, therefore, is used for diagnosis and monitoring of myocardial infarction.

TEST PRINCIPLE

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 activity.



REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1	
Imidazole/Good's buffer	120 mmol/L
Glucose	25 mmol/L
N-Acetylcysteine (NAC)	25 mmol/L
Magnesium acetate	12.5 mmol/L
EDTA-Na ₂	2 mmol/L
NADP	2.5 mmol/L
Hexokinase (HK)	≥ 5 kU/L
Monoclonal antibodies against human CK-M; inhibiting capacity	2500 U/L
Reagent 2	
Imidazole/Good's buffer	90 mmol/L
ADP	10 mmol/L
AMP	28 mmol/L
Glucose-6-Phosphate-Dehydrogenase (G6P-DH)	≥ 15 kU/L
Diadenosine pentaphosphate	50 µmol/L
Creatine phosphate	150 mmol/L

REAGENT PREPARATION

Substrate Start:

Reagents are ready to use.
 Sample Start:

Mix 4 parts of Reagent 1 + 1 part of Reagent 2 (= Working Reagent)

REAGENT STABILITY AND STORAGE

Conditions: protect from light!
 close immediately after use
 avoid contamination

do not freeze the reagents!

Substrate Start:

Storage: at 2 – 8 °C
 Stability: up to the indicated expiration date

Sample Start (Working Reagent):

Stability: at 2 – 8 °C 2 weeks
 at 15 – 25 °C 24 hours

The working reagent must be protected from light!

SAMPLE STABILITY AND STORAGE

Serum, Plasma Stability ⁽⁸⁾: at 20 – 25 °C 2 days
 at 4 – 8 °C 7 days
 at - 20 °C 4 weeks

Discard contaminated specimens. Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
 General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate Start

Pipette into test tubes	Blank	Sample/Calibr.
Sample/Calibrator	-	50 µl
Dist. water	50 µl	-
Reagent 1	1000 µl	1000 µl
Mix. Incubate for approximately 3 minutes. Then add:		
Reagent 2	250 µl	250 µl
Mix. Read initial absorbance after 2 min at 37 °C and start a stopwatch. Read absorbance again after exactly 1, 2, 3, 4 and 5 min. at 37°C		
$\Delta A/\text{min} = [\Delta A/\text{min sample/calibrator}] - [\Delta A/\text{min blank}]$		

Sample Start

Pipette into test tubes	Blank	Sample/Calibr.
Sample/Calibrator	-	40 µl
Dist. water	40 µl	-
Working reagent	1000 µl	1000 µl
Mix. Read initial absorbance after 5 min. at 37°C and start a stopwatch. Read absorbance again after exactly 1, 2, 3, 4 and 5 min. at 37°C		
$\Delta A/\text{min} = [\Delta A/\text{min sample/calibrator}] - [\Delta A/\text{min blank}]$		

CALCULATION

With factor: (light path 1 cm)

CK-MB [U/L] = $\Delta A/\text{min} \times \text{Factor}$

Factor for 340 nm 8254

Factor for 334 nm 8414

With calibrator :

CK-MB [U/L] = $\frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{Conc. Cal [U/L]}$

UNIT CONVERSION

U/L x 0.01667 = µkatal/L

REFERENCE RANGES

The risk of myocardial infarction is high if the following three conditions are fulfilled [6]:

1. CK (men) > 190 U/L (3.12 µkat/L)*
 CK (women) > 167 U/L (2.87 µkat/L)*
2. CK-MB > 24 U/L (0.40 µkat/L)*
3. CK-MB activity is between 6 and 25% of total CK activity.
 * calculated using temperature conversion factor 2.38 (25°C → 37°C)



If myocardial infarction is suspected and the conditions are not fulfilled, the infarction may be fresh. In this case the measurements should be repeated after 4 hours with fresh samples.

In healthy individuals different values are found depending on race and age [6,7].

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary. For diagnostic purposes CK values should always be assessed in conjunction with the anamnesis, the clinical examination and other findings.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

The test has been developed to determine CK-MB activities up to 2000 U/L. If that value is exceeded, samples should be diluted with NaCl solution (9 g/L) and reassayed, multiplying the result by the dilution factor.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 2 U/L

PRECISION (at 37 °C)

Intra-assay, n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	26.7	0.70	2.61
Sample 2	46.6	0.85	1.82
Sample 3	106	1.03	0.97

Inter-assay, n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	28.2	1.05	3.72
Sample 2	52.7	1.66	3.15
Sample 3	109	2.32	1.13

SPECIFICITY/INTERFERENCES

no interference up to:

ascorbic acid 30 mg/dL

conj. and unconj. bilirubin 25 mg/dL

triglycerides 900 mg/dL

hemoglobin interferes at a concentration of 25 mg/dL.

For further information on interfering substances refer to Young DS [9].

METHOD COMPARISON

A comparison between this CK-MB (y) and a commercially available test (x) using 90 samples gave following results:

$$y = 1.00x + 2.08 \text{ U/L}; r = 1.00.$$

QUALITY CONTROL

Control sera containing non-human CK-MB fractions are not suitable to be applied with this test due to the monoclonal antibody used in the reagent. Please take care to use controls containing exclusively human CK-MB.

Each laboratory should establish corrective action in case of deviations in control recovery.

CALIBRATION

The use of a CK-MB Calibrator is optional. Calibrators containing non-human CK-MB fractions are not suitable to be applied with this test due to the monoclonal antibody used in the reagent. Please take care to use calibrators containing exclusively human CK-MB.

WARNINGS AND PRECAUTIONS

- Reagent 1 and 2: Danger
H360D: May damage the unborn child.

P201: Obtain special instructions before use.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P308+P313: If exposed or concerned: Get medical advice/attention.

- The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
- In very rare cases, samples of patients with gammopathy might give falsified results [10].
- Sulfasalazine and sulfapyridine medication may lead to false results in patient samples. Blood collection must be done before drug administration.
- Heterophile antibodies in patient samples may cause falsified results.
- Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
- For diagnostic purposes, the results should always be assessed with the patients' medical history, clinical examinations and other findings.
- For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements


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Symbols used on labels and packaging

 = In vitro diagnostic medical device

REF = Catalog Number

 = Lot Number

 = Manufacturer



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 = Expiration date

 = Temperature limitation

 = Instruction for use

