CK MB FS

CK MB Reagent Test Kit

Intended Use
Diagnostic reagent for in vitro quantitative determination of CK MB in human serum or plasma on photometric analyzers.

Reagent Kits

Item Code	Packsize	
116419934840	R1:4 x 20 mL	R2:2 x 10 mL
116419934841	R1:2 x 20 mL	R2:1 x 10 mL

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 the human body in dimeric forms as CK-MM, CK-MB, CK-BB and as macro-enzyme. 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.

Method
Optimized UV test according to DGKC (German Society of Clinical Chemistry) and IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) for CK with inhibition of CK-M isoenzymes by monoclonal antibodies.

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.

Reaction principle

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

Glucose + ATP $\xrightarrow{HK}$ Glucose-6-phosphate + ADP

Glucose-6-phosphate + $NADP^+$ $\xrightarrow{G6P\text{-}DH}$ 6-Phosphogluconolactone + NADPH + H^+

CK-MB DS
In samples with low CK-MB concentrations the measuring signals are rather low. The supplementary reagent CK-MB DS produces an additional reaction step which duplicates the measuring signal and, therefore, leads to an improvement of the precision and sensitivity:

6-Phospho-glucono lactone $\xrightarrow{PGL}$ 6-Phospho-gluconate

6-Phospho-gluconate + $NADP^+$ $\xrightarrow{6\text{-}PGDH}$ Ribulose-5-phosphate + CO_2 + NADPH + H^+

Reagents
Components and Concentrations

R1	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
R2	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

Storage Instructions and Reagent Stability
The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and if contamination is avoided. Do not freeze the reagents!

Traceability
The reagent is traceable to IFCC reference method.

Warnings and Precautions
1. 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.
2. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. In very rare cases, samples of patients with gammopathy might give falsified results [10].
4. Sulfasalazine and sulfapyridine medication may lead to false results in patient samples. Blood collection must be done before drug administration.
5. Heterophile antibodies in patient samples may cause falsified results.
6. 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 patient's medical history, clinical examinations and other findings.
7. For professional use only!

Waste Management
Please refer to local legal requirements.

Reagent Preparation
Substrate Start
The reagents are ready to use.
For the determination with CK-MB DS:
Mix 1 part of CK-MB DS with 20 parts of reagent 1.
Use mixture as described for reagent R1.
Stability in premixed R1:

6 days	at	2 – 8°C
24 hours	at	15 – 25°C

Sample Start
(without CK-MB DS)
Mix 4 parts of R1 + 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = mono reagent
Stability:

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

The mono reagent must be protected from light.

Materials required but not provided
NaCl solution 9 g/L
General laboratory equipment

Specimen
Serum, Plasma
Stability [8]:

2 days	at	20 – 25°C
7 days	at	4 – 8°C
4 weeks	at	–20°C

Discard contaminated specimens! Freeze only once!

Assay Procedure
Application sheets for automated systems, especially SYS200/400 are available on request.

Wavelength	340 nm, Hg 334 nm
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

Substrate Start

	Blank	Sample or calibrator
Sample or calibrator	-	50 µL
Dist. water	50 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incubate for approx. 3 min., then add:		
Reagent 2	250 µL	250 µL
Mix, read absorbance after 2 min. and start the stopwatch. Read absorbance again after 1, 2, 3, 4 and 5 min.		

Sample Start

	Blank	Sample or calibrator
Sample or calibrator		40 µL
Dist. water	40 µL	
Mono reagent	1000 µL	1000 µL
Mix, read absorbance after 5 min. and start the stopwatch. Read absorbance again after 1, 2, 3, 4 and 5 min.		

Calculation
With factor
From absorbance readings calculate DA/min and multiply by the corresponding factor from the table below:

DA/min x factor = CK-MB activity [U/L]

	without CK-MB DS	***with CK-MB DS***
340 nm	8254	4127
334 nm	8414	4207

With calibrator

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

Conversion factor
CKMB [U/L] x 0.0167 = CKMB [µkat/L]

Calibrators and Controls
For calibration of automated photometric systems, DiaSys TruCal CK-MB calibrator is recommended. The assigned values of the calibrator have been made traceable to the molar extinction coefficient. Control sera and 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 controls and calibrators containing exclusively human CK-MB. For internal quality control we recommend DiaSys TruLab N and P controls to be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

Performance Characteristics
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) to activities of less than 2000 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. Hemoglobin interferes even in minimum concentrations as from 25 mg/dL. For further information on interfering substances refer to Young DS [9].

Sensitivity/Limit of Detection
The lower limit of detection is 2 U/L.

Precision

Intra-assay precision 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 precision 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	2.13

Method Comparison
A comparison of DiaSys CK-MB FS (y) with a commercially available test (x) using 90 samples gave following results:
y = 1.00 x + 2.08 U/L; r = 1.00

Reference Range
Myocardial infarction: The risk of myocardial infarction is high if the following three conditions are fulfilled [6]:
1. CK (Men) > 190 U/L (3.17 µkat/L)**
   CK (Women) > 167 U/L (2.78 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.

Clinical Interpretation
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.

Limitations
The result interference can be possible with patient samples exceeding Triglyceride 900 mg/dL.

Literature

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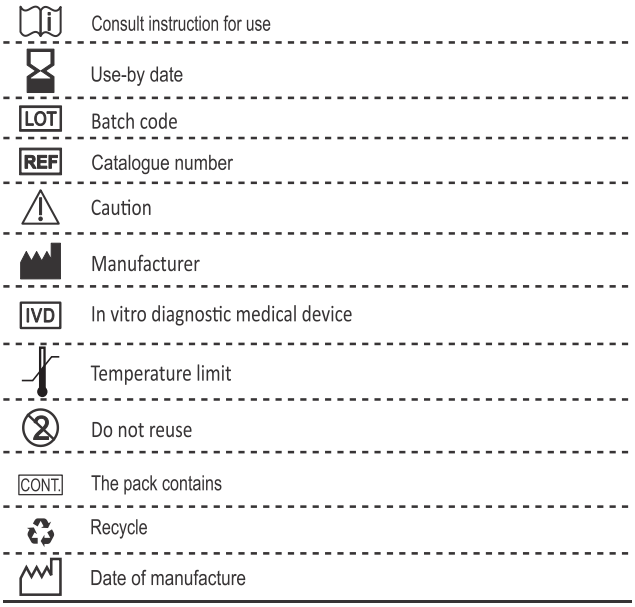
Notes on Symbols and Marks



ISO 9001, ISO 13485 and ICMED 13485 Certified Company

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Revision No. :03
Feb. : 2022

IN/0017 v03