DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
Hondastrasse, Objekt M55, IZ NOE-Sued, 2351 Wiener Neudorf, Austria,
Phone: +43 (0) 2236 660910-0, Fax: +43 (0) 2236 660910-30, e-mail: office@dialab.at

Liquid Reagents – ready to use

CK-MB

opt. DGKC / IFCC

2 Reagents

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

Ref.No.	Kit Size	Content
D10582B	**1 x 1 L**	1 x 0.8 L R1 + 1 x 0.2 L R2
D10585	**5 x 100 mL**	4 x 100 mL R1 + 1 x 100 mL R2
D10586	**5 x 50 mL**	4 x 50 mL R1 + 1 x 50 mL R2
D10587	**5 x 25 mL**	4 x 25 mL R1 + 1 x 25 mL R2
D10588	**5 x 10 mL**	4 x 10 mL R1 + 1 x 10 mL R2
D35911	**5 x 50 mL**	4 x 50 mL R1 + 2 x 25 mL R2
D0450917	**5 x 62.5 mL**	4 x 62.5 mL R1 + 1 x 62.5 mL R2
DA1018	**5 x 20 mL**	4 x 20 mL R1 + 1 x 20 mL R2
DT1018	**5 x 20 mL**	4 x 20 mL R1 + 1 x 20 mL R2
DK1018	**5 x 50 mL**	4 x 50 mL R1 + 1 x 50 mL R2
DE1818	**2 x 62.5 mL**	2 x 50 mL R1 + 2 x 12.5 mL R2

Additionally offered (optional):

D13595	5 x 1 mL	Calibrator	Diacal CK-MB
D13595SV	1 x 1 mL	Calibrator	Diacal CK-MB
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98482SV	1 x 5 mL	Control abnormal	Diacon P

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 cardic 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.

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

Glucose + ATP $\xrightarrow{HK}$ Glucose-6-Phosphate (G-6-P) + ADP

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

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 ΔA/min = [ΔA/min sample/calibrator] – [ΔA/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 ΔA/min = [ΔA/min sample/calibrator] – [ΔA/min blank]		

CALCULATION

With factor: (light path 1 cm)
CK-MB [U/L] = ΔA/min x Factor

Factor for 340 nm 8254
Factor for 334 nm 8414

With calibrator :

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

UNIT CONVERSION

U/L x 0.01667 = µkatal/L

DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
Hondastrasse, Objekt M55, IZ NOE-Sued, 2351 Wiener Neudorf, Austria,
Phone: +43 (0) 2236 660910-0, Fax: +43 (0) 2236 660910-30, e-mail: office@dialab.at

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	2.13

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 Dialab CK-MB (y) and a commercially available test (x) using 90 samples gave following results:
y = 1.00 x + 2.08 U/l; r= 1.00.

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.

We recommend the Dialab CK-MB calibration serum **Diacal CK-MB**. The assigned values of this calibrator have been made traceable to the molar extinction coefficient.

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.

We recommend the Dialab serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range).

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

AUTOMATION

Special adaptations for automated analyzers can be made on request.

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.
7. For diagnostic purposes, the results should always be assessed with the patients' medical history, clinical examinations and other findings.
8. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements

REFERENCES

1. Stein W. Creatine kinase (total activity), creatine kinase isoenzymes and variants. In: Thomas L, ed. Clinical laboratory diagnostics. Frankfurt: TH-Books Verlagsgesellschaft;1998.p.71-80.
2. Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 617-721.
3. Würzburg U, Hennrich N, Orth HD, Lang H. Quantitative determination of creatine kinase isoenzyme catalytic concentrations in serum using immunological methods. J Clin Chem Clin Biochem 1977;15:131-7.
4. Recommendations of the German Society for Clinical Chemistry. Standardization of methods for the estimation of enzyme activities in biological fluids: Standard method for the determination of creatine kinase activity. J Clin Chem Clin Biochem 1977;15:255-60.
5. Schumann G, Bonora R, Ceriotti F, Férard G et al. IFCC primary reference procedure for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 5: Reference procedure for the measurement of catalytic concentration of creatine kinase. Clin Chem Lab Med 2002; 40:635-42.
6. Stein W. Strategie der klinisch-chemischen Diagnostik des frischen Myokardinalfarkts. Med Welt 1985:36:572-7
7. Myocardial infarction redefined – a consensus document of the Joint European society of Cardiology / America College of Cardiology Committee for the redefinition of myocardial infarction. Eur Heart J 2000; 21:1502-13.
8. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p.24-5.
9. Young DS. Effects of Drugs on Clinical laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
10. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.

CE 2°C 8°C IVD