Creatinine Kinase

DiaSystem Scandinavia AB

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

Order Information

Cat. No.	Kit size						
01 00017 70 04 0125	R1	5 x	20 mL	+	R2	1 x	25 mL
01 00017 70 04 0500	R1	5 x	80 mL	+	R2	1 x	100 mL
01 00017 70 10 0180	R1	4 x	36 mL	+	R2	4 x	9 mL
01 00017 70 02 0180	R1	4 x	36 mL	+	R2	4 x	9 mL
CDT-CK	R1	3 x	30 mL	+	R2	2 x	11.3 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 serum in dimeric form as CK-MM, CK-MB, CK-BB and as macroenzyme. Elevated CK values are observed in cardiac muscle damages and in skeletal muscle diseases. Measurement of CK is used especially in conjunction with CK-MB for diagnosis and monitoring of myocardial infarction.

Method

Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) and DGKC (German Society of Clinical Chemistry)

Principle

Creatine phosphate + ADP <CK> Creatine + ATP

Glucose + ATP <HK> Glucose-6-phosphate + ADP

Glucose-6-phosphate + $NADP^+$ <G6P-DH> Gluconate-6-phosphate + NADPH + H^+

Reagents

Components and Concentrations

R1	Imidazole	pH 6.0	60 mmol/L
	Glucose		27 mmol/L
	N-Acetylcysteine (NAC)		27 mmol/L
	Magnesium acetate		14 mmol/L
	EDTA-Na_2		2 mmol/L
	NADP		2.7 mmol/L
	Hexokinase (HK)		≥ 5 kU/L
R2	Imidazole		160 mmol/L
	ADP		11 mmol/L
	AMP		28 mmol/L
	Diadenosine pentaphosphate		55 µmol/L
	Glucose-6-phosphate dehydrogenase (G6P-DH)		≥ 14 kU/L
	EDTA-Na_2		2 mmol/L
	Creatine phosphate		160 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 contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. Reagent R2 is toxic. R61: May cause harm to the unborn child. S53: Avoid exposure-obtain special instructions before use. S28: After contact with skin, wash immediately with plenty of water. S29: Do not empty into drains. S36/37: Wear suitable protective clothing and gloves. S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
2. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
4. Reagent 2 contains animal material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
5. In very rare cases, samples of patients with gammopathy might give falsified results [9].
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 patients' 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.

Sample start

Mix 4 parts of R1 + 1 part of R2
(e. g. 20 mL R1 + 5 mL R2) = mono reagent

Stability:	3 weeks	at	2 – 8 °C
	2 days	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, heparin plasma or EDTA plasma

Stability [4]:	2 days	at	20 - 25 °C
	7 days	at	4 - 8 °C
	4 weeks (in the dark)	at	- 20 °C

Only freeze once! Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	340 nm, Hg 365 nm, Hg 334 nm
Optical path	1 cm
Temperature	37 °C
Measurement	Against reagent blank

Substrate start

	Blank	Sample
Sample/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 and 3 min.		

ΔA/min = ΔA/min sample/calibrator

Sample start

	Blank	Sample
Sample/Calibrator		40 µL
Dist. water	40 µL	
Mono-reagent	1000 µL	1000 µL
Mix, read absorbance after 3 min and start the stopwatch.		
Read absorbance again after 1, 2 and 3 min.		

ΔA/min = ΔA/min sample/calibrator

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Calculation

With factor

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

ΔA/min x factor = CK activity [U/L]

340 nm	4127
334 nm	4207
365 nm	7429

With calibrator

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

Conversion factor

CK [U/L] x 0.0167 = CK [µkat/L]

Calibrators and Controls

For the calibration of automated photometric systems, DiaSystem UniCal U calibrator is recommended. This method has been standardized against the original IFCC formulation. For internal quality control DiaSystem UniLab N and P controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
UniCal CC	06 00122 70 04 0018	6 x 3 mL
UniLab N	07 00123 70 05 0030	6 x 5 mL
UniLab P	07 00124 70 05 0030	6 x 5 mL

Performance Characteristics

Measuring range

On automated systems the test is suitable for the determination of CK activities up to 1100 U/L.
In case of a manual procedure, the test is suitable for CK activities which correspond to a maximum of ΔA/min of 0.25 at 334 and 340 nm or 0.14 at 365 nm.
If such values are exceeded the samples should be diluted 1 + 9 with NaCl solution (9 g/L) and results multiplied by 10.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 200 mg/dL and lipemia up to 2000 mg/dL triglycerides. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 1 U/L.

Precision

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	159	3.18	2.00
Sample 2	220	1.54	0.70
Sample 3	508	3.69	0.73

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	49.5	1.05	2.12
Sample 2	157	1.63	1.04
Sample 3	228	2.31	1.01

Method Comparison

A comparison of DiaSystem Creatine Kinase (y) with the IFCC reference reagent (x) using 51 samples gave following results:
y = 0.997 x - 0.249 U/L; r= 0.999.

A comparison of DiaSystem Creatine Kinase (y) with a commercially available test (x) using 51 samples gave following results:
y = 1.031 x + 0.059 U/L; r= 1.000.

Reference Range

Adults [6]

Women	< 145 U/L	< 2.42 µkat/L
Men	< 171 U/L	< 2.85 µkat/L

These reference ranges ensure high diagnostic sensitivity. The diagnostic specificity is low; however, it can be improved by additional measurement of CK-MB.
Myocardial infarction: The risk of myocardial infarction is high if following three conditions are fulfilled [7]:

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.

In healthy individuals different values are found depending on race and age [7, 8].

Children [1]

Umbilical cord blood	175 - 402 U/L	2.92 – 6.70 µkat/L
Newborns	468 - 1200 U/L	7.80 – 20.0 µkat/L
≤ 5 days	195 - 700 U/L	3.25 – 11.7 µkat/L
< 6 months	41 - 330 U/L	0.68 – 5.50 µkat/L
> 6 months	24 - 229 U/L	0.40 – 3.82 µkat/L

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.

Literature

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