CK-NAC FS

CK NAC Reagent Test Kit

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

Reagent Kits

Item Code	Packsize	
116019934840	R1: 4 x 20 mL	R2: 2 x 10 mL
116019934841	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 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 $\xleftrightarrow{CK}$ Creatine + ATP

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

Glucose-6-phosphate + $NADP^+$ $\xleftrightarrow{G6P\text{-}DH}$ 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	pH 9.0	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 1: 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. Reagent 2: Danger. H315 Causes skin irritation. H319 Causes serious eye irritation. H360D May damage the unborn child. P201 Obtain special instructions before use. P280 Wear protective gloves/ protective clothing/eye protection/face protection. P302+P352 If on skin: Wash with plenty of water/soap. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P308+P313 If exposed or concerned: Get medical advice/attention.
3. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
4. Reagent 2 contains biological 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

Traceability:
The method is traceable to IFCC reference method.

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 and 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, especially SYS200/400 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 stopwatch. Read absorbance again after 1, 2 and 3 min.		

DA/min = DA/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 stopwatch. Read absorbance again after 1, 2 and 3 min.		

DA/min = DA/min sample/calibrator

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

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

340 nm	4127
334 nm	4207
365 nm	7429

With calibrator

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

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

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

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	145	0.96	0.65
Sample 2	229	2.95	1.28

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	134	1.11	0.82
Sample 3	426	4.60	1.08

Method Comparison
A comparison of DiaSys CK-NAC FS (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 DiaSys CK-NAC FS (y) with a commercially available test (x) using 51 samples gave following results:
y = 1.03 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.

Clinical Interpretation
Serum creatine kinase (CK) activity may increase in patients with acute cerebrovascular disease or neurosurgical intervention and with cerebral ischemia as well as in nearly all patients when injury, inflammation, or necrosis of skeletal or heart muscle.

Limitation
Interference in the results can be seen with Patient samples with lipemia above 2000 mg/dL triglycerides.

Literature

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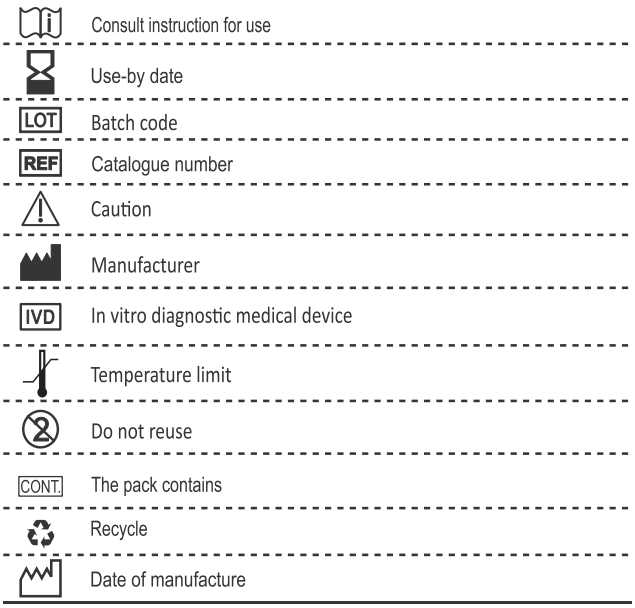
Notes on Symbols and Marks



ISO 9001, ISO 13485 and ICMED 13485 Certified Company

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