ISE S.r.l.
CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

CK NAC - Instruction for use

CE

IVD

R1: 2 x 18 mL REF R3330000017
R2: 2 x 4,5 mL

Diagnostic reagent for quantitative in vitro determination of creatinkinase (CK-NAC) in human serum or plasma on photometric systems

TEST PARAMETERS

Method:	UV, Kinetic, Increasing Reaction, **o**pt. DGKC
Wavelength:	340 nm, Hg 334 nm, Hg 365 nm
Temperature:	37°C
Sample:	Serum, EDTA-plasma, heparin plasma
Linearity:	up to 1100 U/L
Sensitivity:	The lower limit of detection is 1 U/L.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1:	
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
Reagent 2:	
Imidazole, pH 9.0	160 mmol/L
ADP	11 mmol/L
AMP	28 mmol/L
Diadenosine pentaphosphate	55 µmol/L
Glucose-6-phosphate dehydrogenaase (G6P-DH)	≥ 14 kU/L
EDTA-Na_2	2 mmol/L
Creatine phosphate	160 mmol/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. 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.

TEST PRINCIPLE

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

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

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

REAGENT PREPARATION

Substrate Start:
Reagents are ready for use.

Sample Start:
Mix 4 parts of Reagent 1 with 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 expiration date

Sample Start (Working Reagent):

Stability:	at 2 – 8°C	3 weeks
	at 15 – 25°C	2 days

The working reagent must be protected from light!

SAMPLE STABILITY AND STORAGE

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

Only freeze once! Discard contaminated specimens.

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/Cal.
Sample	-	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 and 3 min. at 37°C. ΔA/min = [ΔA/min sample/calibrator] – [ΔA/min blank]		

Sample Start

Pipette into test tubes:	Blank	Sample/Cal.
Sample	-	40 µL
Dist. water	40 µL	-
Working Reagent	1000 µL	1000 µL
Mix. Read initial absorbance after 3 min. at 37°C and start a stopwatch. Read absorbance again after exactly 1, 2 and 3 min. at 37°C. ΔA/min = [ΔA/min sample/calibrator] – [ΔA/min blank]		

CALCULATION

With factor: (light path 1 cm)
CK-NAC [U/L] = ΔA/min x factor
Factors (37°C):
Factor for : 340 nm = 4127
Factor for : 334 nm = 4207
Factor for : 365 nm = 7429
With calibrator :

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

UNIT CONVERSION

U/L x 0,01667 = µkatal/L

REFERENCE RANGES

Adults: [6]

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

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

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Revision A R3330000017 11-2018

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

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 [7,8].
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

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 to determine CK activities which correspond to a maximal ΔA/min of 0.25 at 340 nm and 334 nm or 0.14 at 365 nm.
If these values are exceeded, the sample should be diluted 1+9 with NaCl solution (9 g/L sodium chloride in dist. water) and results multiplied by 10.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 1 U/L

PRECISION (at 37 °C)

Intra-assay, 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, 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

SPECIFICITY/INTERFERENCES

no interference up to:

ascorbic acid	30 mg/dL
bilirubin	40 mg/dL
hemoglobin	200 mg/dL
triglycerides	2000 mg/dL

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

A comparison of the this CK-NAC (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 between Dialab CK-NAC (y) and a commercially available test (x) using 51 samples gave following results:
y = 1.031 x + 0.059 U/L; r = 1.000.

QUALITY CONTROL

All control sera with CK-NAC values determined by this method can be used.
Each laboratory should establish corrective action in case of deviations in control recovery.

CALIBRATION

The use of a CK-NAC Calibrator is optional.

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.
   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.
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

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R1: 2 x 18 mL REF R3330000017
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Symbols used on labels and packaging

IVD = In vitro diagnostic medical device

REF = Catalog Number

LOT = Lot Number

= Manufacturer

= Expiration date

= Temperature limitation

= Instruction for use

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