

Liquid Reagents – ready to use

# CK-NAC (Creatine Kinase - NAC)

opt. DGKC / IFCC

2 Reagents

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

Ref.No.	Kit Size	Content
DIA010071	62.5 ml	2x25 ml R1 + 1x12.5 ml R2
DIA010072	5 x 20 ml	4x20 ml R1 + 1x20 ml R2
DIA010073	5 x 25 ml	4x25 ml R1 + 1x25 ml R2

Additionally offered:

DIA040012	1 x 3 mL	Diacal Auto (Calibrator)
DIA030012	1 x 5 mL	Diacon N (Control Normal)
DIA030022	1 x 5 mL	Diacon P (Control Abnormal)

## TEST PARAMETERS

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

## REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
<b>Reagent 1:</b>		
Imidazole, pH 6.0	60	mmol/L
Glucose	27	mmol/L
N-Acetylcysteine (NAC)	27	mmol/L
Magnesium acetate	14	mmol/L
EDTA-Na <sub>2</sub>	2	mmol/L
NADP	2.7	mmol/L
Hexokinase (HK)	≥ 5	kU/L
<b>Reagent 2:</b>		
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 <sub>2</sub>	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  $\xrightarrow{\text{CK}}$  Creatine + ATP

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

G-6-P + NADP<sup>+</sup>  $\xrightarrow{\text{G6P-DH}}$  Gluconate-6-phosphate + NADPH + H<sup>+</sup>

## 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.		
$\Delta A/\text{min} = [\Delta A/\text{min sample/calibrator}] - [\Delta A/\text{min blank}]$		

### 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.		
$\Delta A/\text{min} = [\Delta A/\text{min sample/calibrator}] - [\Delta A/\text{min blank}]$		

## CALCULATION

With factor: (light path 1 cm)

CK-NAC [U/L] =  $\Delta A/\text{min} \times \text{factor}$

Factors (37°C):

Factor for : 340 nm = 4127

Factor for : 334 nm = 4207

Factor for : 365 nm = 7429

With calibrator :

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



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  $\mu$ kat/L)\*  
CK (women) > 167 U/L (2.87  $\mu$ kat/L)\*
2. CK-MB > 24 U/L (0.40  $\mu$ kat/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  $\Delta A/\text{min}$  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	157	1.63	1.04
Sample 2	228	2.31	1.01
Sample 3	507	4.09	0.81

### SPECIFICITY/INTERFERENCES

No interference up to:

<b>Ascorbic acid</b>	30 mg/dL
<b>Bilirubin</b>	40 mg/dL
<b>Hemoglobin</b>	200 mg/dL
<b>Triglycerides</b>	2000 mg/dL

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

### METHOD COMPARISON

A comparison of the Diagnostica CK-NAC (y) with the IFCC reference reagent (x) using 51 samples gave following results:

$$y = 0.997 x - 0.249 \text{ U/L}; r = 0.999.$$

A comparison between Diagnostica CK-NAC (y) and a commercially available test (x) using 51 samples gave following results:

$$y = 1.031 x + 0.059 \text{ U/L}; r = 1.000.$$

### QUALITY CONTROL

All control sera with CK-NAC values determined by this method can be used. We recommend the Diagnostica 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.

### CALIBRATION

The use of a CK-NAC Calibrator is optional.

We recommend the Diagnostica multi calibration serum **Diacal Auto**. This method has been standardized against the original IFCC formulation.

### AUTOMATION

Special applications for automated analysers can be made on request.

### WARNINGS AND PRECAUTIONS

1. Reagent 1: Danger.  
H360: May damage fertility or 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.

H360D: May damage the unborn child.

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

