CREATINE KINASE – Nac (CK – Nac)

Diagnostic reagent for the in-vitro quantitative determination of Creatine Kinase Nac (CK-Nac) in human serum on manual systems.

REF: V/CK05.005	25 test	REF: V/CK05.010	50 test

CLINICAL SIGNIFICANCE

Creatine kinase (CK) is an enzyme which is contained in heart, brain and skeletal muscles. Thus, an increase of circulating level of CK may be associated to myocardial infarction, acute cerebrovascular disease, trauma or diseases of skeletal muscles. After a myocardial infarct, CK level begins raising between 4th and 6th hour after first acute symptoms, reaching the peak between 18th and 30th hour and coming back to normal values during the 3rd day. CK is present in three different iso enzymatic forms, which could be separated by electrophoresis or column chromatography; each form is originated in different body tissues, paying off their diagnostic determinations. The formula of present reagent is based on the Deutsche Gesellschaft für Klinische Chemie (DGKC) and the International Federation of Clinical Chemistry (IFCC) recommendations.

METHOD PRINCIPLE (2)

According to the recommendations of (IFCC).
Creatine kinase (CK) catalyzes the phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine. The catalytic concentration is determined from the rate of NADPH formation, measured at 340 nm, by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH) coupled reactions.

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

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

$$\text{Glucose-6-phosphate} + \text{NADP+} \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate} + \text{NADPH} + \text{H+}$$

REAGENT COMPOSITION

Reagent (R1): Buffer / Coenzyme (pH 6.7)	
Imidazol	125mmol/L
D-Glucose	25mmol/L
N-Acetyl-L-Cysteine	25 mmol/L
Magnesium acetate	12.5mmol/L
NADP	2.5 mmol/L
EDTA	2 mmol/L
Reagent (R2): Enzymes	
ADP	15.2mmol/L
AMP	25 mmol/L
P1,P5-di (adenosine-5'-) penta-phosphate	103 mmol/L
Glucose-6-phosphate Dehydrogenase (G6PDH)	9 KU/L
Creatine phosphate	250 mmol/L
Hexokinase (HK)	3 KU/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment.

For further information, refer to the **Lab.Vie**. CK-Nac reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie. CK-Nac working solution is prepared by combining four volume of R1 with one volume of R2 and mix gently, e.g.4.0 ml R1 +1.0 ml R2. **Lab.Vie**. CK-Nac working solution is stable for 2 weeks at 2-8°C away from light sources. **Lab.Vie**. CK-Nac reagents are stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8°C.

Deterioration
The **Lab.Vie**. CK-Nac reagent is normally clear when protected from light and contaminations prevented during their use. Do not use **Lab.Vie**. CK-Nac reagent if it is turbid or presence of foreign particles.

SPECIMEN COLLECTION AND PRESERVATION (3)

Serum or plasma
Serum free of haemolysis or heparin plasma. Stability 2 days at 20-25 °C, 7 days at 2-8°C, 4 weeks at -20°C protected from light.

SYSTEM PARAMETERS

Wavelength	340 nm (334 – 365 nm)
Optical path	1 cm
Assay type	Kinetic
Direction	Increase
Sample Reagent Ratio	1:25
e.g.: Reagent volume	1 ml
Sample volume	40 µl
Temperature	37 °C
Equilibration time	60 Seconds
Read Time	1 to 3 minutes
Reading interval	1 minute
No. of Reading	3
Zero adjustment	against air
Sensitivity	1 U/L
Linearity	2000 U/L

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet and Thermostatized cuvette
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	macro	Semi-micro
Working Solution	1 ml	0.5 ml
Specimen	40 µl	20 µl

Read initial absorbance after 60 seconds, and start timer immediately. Read again after 1, 2 and 3 minutes and determine the mean absorbance change per minute (ΔA/min).

CALCULATION

CK-Nac U/L = ΔA/min x 4127

NB.: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per liter of sample (U/L).

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie**. technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean U/L	147	494	145	485
SD. U/L	1.23	3.60	2.91	8.97
CV. %	0.84	0.73	2.01	1.85

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from **Lab.Vie**. CK-Nac reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.983.
Sensitivity
When run as recommended, the minimum detection limit of the assay is 1.0 U/L.
Linearity
The reaction is linear up to CK-Nac concentration of 2000 U/l; specimens showing higher concentration should be diluted 1+2 using physiological saline and repeat the assay (result×3).

INTERFERING SUBSTANCES (2,4)

Haemolysis
No significant interference from haemoglobin up to < 5 g/L.
Icterus
No significant interference from free and conjugated bilirubin up to levels < 20 mg/dL.
lipemia
No significant interference from Lipids up to levels < 7 mmol/L.
Others
Other drugs and substances may interfere.

EXPECTED VALUES (3)

Serum and plasma	U/L
Men	24 - 204
women	24 - 173

DYNAMIC RANGE

1 - 2000 U/L; It is recommended that each laboratory should establish its own reference range.

REFERENCES

1. Tietz N. W., Textbook of Clinical Chemistry, 3rd edition. Burtis CA, Ashwood ER. WB Saunders Co., 1999 p 664-667, 1185-1190.

2. Friedman and Young. Effects of disease on clinical laboratory tests, 3th ed. AACC Press, 1997.
3. Tietz N. W., Clinical Guide to Laboratory Tests, 4th Edition, (2006) p 310-315.
4. Urdal P and Landaas S. Clin Chem 1979; 25: 461-465.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number	⚠	Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		