CK-NAC

Diagnostic reagent for determination of CK-NAC (Creatine Kinase-N-acetyl-L-cysteine) concentration.
Liquid. Dual reagents. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Pack
MH-132	75 mL
MH-133	50 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

This test is used for the quantitative determination of creatinine kinase (CK) enzyme activity in human serum and plasma.

GENERAL INFORMATION

Creatine kinase (EC 2.7.3.2; ATP: creatine N-phosphotransferase; CK) is a dimeric enzyme (82 kDa) that catalyzes the reversible phosphorylation of the creatinine (Cr) molecule by adenosine triphosphate (ATP). Physiologically, when muscle contracts, ATP is converted to adenosine diphosphate (ADP) and CK then catalyzes the refosphorylation of ADP to ATP as a phosphorylation reserve. The ideal pH for the forward reaction (Cr+ATP→ADP+CrP) is 9, while the pH for the reverse reaction (CrP+ADP→ATP+Cr) is 6.7. Neutral pH is favorable for ATP formation, while pH 9 is ideal for the formation of CrP, another high-energy compound.[1]

CK activity is highest in striated muscle and cardiac tissue, at 2500 and 550 U/g protein, respectively. Other tissues such as the brain, smooth muscles of the gastrointestinal tract and bladder have significantly less activity, and the liver and erythrocytes are essentially devoid of activity.[1] CK is a dimer composed of two subunits (B and M) each with a molecular weight of about 40 kDa. These subunits are products of loci on chromosomes 14 and 19, sequentially. Since the active form of the enzyme is a dimer, only three different pairs of subunits can exist: BB (or CK-1), MB (or CK-2) and MM (or CK-3). All three of these isoenzyme species are found in the cytosol of the cell or are associated with myofibrillar structures. However, there is a fourth isoenzyme that differs from the others in both immunological and electrophoretic mobility. This isoenzyme (CK-Mt) is located between the inner and outer membranes of mitochondria.

Creatine kinase in serum can also be found in a macromolecular form called macro-CK. Reduced clearance of this atypically high molecular mass enzyme results in abnormally high serum CK activity. Macro-CK exists in two forms: type 1 and 2. Type 1 CK is an immunoglobulin complex, typically CK-BB and often IgG, but other complexes such as CK-MM with IgA have also been characterized. More than 80% of macro-CK type 1 (immunoglobulin-dependent) positive individuals are female.[2] Macro-CK type 2 is oligomeric CK-Mt, with a reported prevalence of 0.5% to 2.6% in hospitalized patients. It is mainly found in adults who are severely ill with malignancy or liver disease and in children with visible tissue distress. The appearance of this form in serum is usually associated with a poor prognosis.[1]

CK detection is the laboratory test preferred in case of suspected muscle damage. When injury, inflammation or necrosis occurs in skeletal (or cardiac) muscle, serum CK activity increases in almost all patients.[1] Increased serum CK activity may be the only symptom of subclinical neuromuscular disorders.[3] Serum CK activity is dramatically increased in all types of muscular dystrophy. In progressive muscular dystrophy (especially Duchenne muscular dystrophy), enzyme activity in serum is at its highest during infancy and childhood (7 to 10 years) and may increase long before the disease becomes clinically evident. Serum CK activity characteristically decreases as patients age and functional muscle mass decreases with disease progression. In 50% to 80% of asymptomatic female carriers of Duchenne dystrophy, there is a 3- to 6-fold increase in CK activity. High CK values [up to 50 times the upper reference limit (URL) in active disease] have been recorded in viral myositis, polymyositis, immune-mediated and other inflammatory myopathies.

However, serum enzyme activity is not increased in neurogenic muscle diseases such as myasthenia gravis, multiple sclerosis, polio and parkinsonism. Very high activity is also found in malignant hyperthermia, a life-threatening inherited condition characterized by high fever and induced by the application of inhalation anesthesia (usually halothane) to the affected individual. Molecular genetic studies have confirmed that the skeletal muscle-type ryanodine receptor is the major malignant hyperthermia locus; more than 70% of families carry a mutation in this gene.[1,4]

In acute rhabdomyolysis caused by crush injury with severe skeletal muscle damage, serum CK activities exceeding 200 times the URL may be found. In this case, very high serum CK, reflecting myoglobinuria and its mechanism of heme-induced renal injury, has been associated with the risk of developing acute kidney injury (AKI). If CK remains below 5000 U/L (approximately 30 times the URL) for the first 3 days after trauma, the probability of developing AKI appears to be low.[5]

A temporary increase in CK may help distinguish generalized tonic-clonic seizures from syncope or psychogenic non-epileptic seizures, but given the moderate sensitivity, the test is

not useful in excluding epileptic seizures.[6] Serum CK activity may also be slightly increased by other direct trauma to muscles, such as intramuscular injection and surgery. Lastly, some drugs can increase serum CK activity when given at pharmacologic doses. Hypothyroidism is a common cause of endocrine myopathy. Up to 60% of hypothyroid patients show a mean increase in CK activity five times greater than the URL; therefore, one of the conditions of thyroid hypofunction to consider is patients with unexplained persistent increases in serum CK. Changes in serum CK and MB isoenzyme levels after acute MI have been the basis of diagnosis for many years. However, it is now clinically more appropriate to use heart-specific troponin I or T. As a final point, a sixfold increase in maternal serum CK activity occurs during physiologic labor. Surgical intervention during labor can further increase serum CK activity.[1]

TEST PRINCIPLE

N-Acetyl-L-cysteine (NAC)

It is a standard method optimized according to the recommendations of the German Society for Clinical Chemistry and Laboratory (Deutsche Gesellschaft für Klinische Chemie und Laboratoriumsmedizin) (DGKL).

Creatine kinase catalyzes the transfer of a high-energy phosphate group from creatine phosphate to ADP. The ATP released from this reaction is then used to link glucose to the phosphoryl group in the presence of hexokinase to produce glucose-6-phosphate (G-6-P). G-6-P is oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) and is also reduced to nicotinamide adenine dinucleotide phosphate (NADP), reduction nicotinamide adenine dinucleotide phosphate (NADPH). NADPH formation is monitored at 340 nm and is directly proportional to CK activity.

These reactions occur in the presence of N-acetyl-L-cysteine (NAC) as an enzyme reactivator.

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

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

$$Glucose - 6 - Phosphate + NADP^{+} \xrightarrow{G6PD} 6 - Phosphogluconate + NADPH + H^{+}$$

Note 1: Numerous photometric, fluorometric and matched enzyme methods have been developed for the detection of CK activity using a forward (Cr → CrP) or reverse (CrP → Cr) reaction. Currently, the principle of many commercial assay methods for the measurement of total CK activity is based on the reverse reaction, which is approximately six times faster than the forward reaction.[1]

Note 2: The enzyme in serum is relatively unstable; there is loss of activity due to oxidation of the sulfhydryl group in the active site of the enzyme. Activity can be partially restored by incubating the enzyme preparation with sulfhydryl compounds such as N-acetylcysteine, dithiothreitol (Cleland reagent) and glutathione. Currently, the preferred agent for the determination of enzyme activity is N-acetylcysteine, which has the advantage of being a very soluble substance used at a final concentration in the test reagent of approximately 20 mmol/L.[1]

REAGENT COMPONENTS

Reagent 1 & 2

Imidazole	: ≤120 mmol/L
Creatinephosphate	: ≤ 34 mmol/L
D-Glucose	: ≤ 22 mmol/L
N-Acetylcystein	: ≤ 22 mmol/L
Magnesium acetate	: ≤ 12 mmol/L
EDTA	: ≤ 2.2 mmol/L
ADP	: ≤ 2.2 mmol/L
AMP	: ≤ 5.5 mmol/L
Diadenosinpentaphosphate	: ≤ 15 µmol/L
Glucose-6-Phosphate-DH	: > 1.5 kU/L
Hexokinase	: > 2.5 kU/L
NADP	: ≤ 2 mmol/L

Note: The assay is optimized by adding (1) N-acetylcysteine to activate CK, (2) ethylenediaminetetetraacetic acid (EDTA) to bind Ca^{+2} and increase the stability of the reaction mixture, and (3) Diadenosine pentaphosphate (Ap_5A) to inhibit Adenylate kinase to AMP.

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[7]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. For plasma, sample collection tubes with lithium heparin or sodium heparin should be preferred.

Icteric samples must not be used.

CK activity stability in serum and plasma[1]:

8> hours at +20/+25 ºC
48 hours at +2/+8ºC
30 days at -20ºC

Note 1: Samples for CK analysis include serum and heparin plasma. Anticoagulants other than heparin inhibit CK activity and thus should not be used during blood collection.

Note 2: CK activity in serum is relatively unstable and is rapidly lost during storage. Average stabilities are less than 8 hours at

room temperature, 48 hours at 4°C and 1 month at -20°C. For this reason, the serum sample should be refrigerated to 4°C if the sample is not analyzed immediately and stored at -80°C if analysis is delayed for more than 30 days.[1]

Unit Conversion:
U/L x 0.0167 = µkat/L

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a Arcal Auto Calibrator.

Arcal Auto Calibrator
Ref.No: VT-003

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Traceability is provided by ERM-AD455k IFCC Creatine Kinase Isoenzyme MM (CK-MM) material.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control- Lyophilized
Ref.No: VT-001

Arcon P Level 2 Control- Lyophilized
Ref.No: VT-002

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Serum / Plasma[20]

Women	: 29 - 168 U/L
Men	: 30 - 200 U/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval data have been verified by using CLSI EP28-A3c protocol.[8]

PERFORMANCE CHARACTERISTICS

Measuring Interval
According to CLSI EP34-ED1:2018, “Measuring Interval” refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte’s lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[9]

The determined analytic measuring interval for CK-NAC is 5-2000 U/L.

Detection Capability
Limit of Detection (LoD): 3 U/L.

Limit of Quantitation (LoQ): 5 U/L.

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[10]

Linearity
This method shows measurement linearity in the activities up to 2000 U/L. Autoanaylzer’s auto-dilution system can be used if the concentrations have higher values. See device manual for further information.
For the manual dilution procedure, dilute the sample 1:10 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[11]

Precision
Running system has been developed according to 20x2x2 “The Single Site” protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.
According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[12]

Repeatability (Within Run) and Repeatability (Day to Day) SD and CV% values of CK-NAC have been given in the table 1 and 2 respectively.

Table 1. CK-NAC Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD*	CV%	n
108 U/L	0.59	0.55	80
296 U/L	1.61	0.54	80

***SD: Standard Deviation**

Note: This working system has been named “Within-Run Precision” in the previous CLSI - EP05-A2 manual.[13]

Table 2. CK-NAC Repeatibility (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
108 U/L	3.73	3.45	80
296 U/L	10.4	3.51	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[13]

Method Comparison

As a result of the statistical evaluation of the method comparison data:[14]
r=0.998

Passing-Bablock equation:
y= 0.99x – 1.7 U/L

Interference

Endogenous interferant and analyte concentrations that have been used in the CK-NAC scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[15,16]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from CK-NAC interference scanning test is appropriate, is determined as ±10%.[17]

In CK-NAC test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte. Due to high interference with icteric samples, such samples must be rejected for CK-NAC testing.

Interferant-Concentration	CK-NAC Target (U/L)	N*	Observed Recovery %
Hemoglobin 1260 mg/dL	106	3*	98
Lipemia 2149 mg/dL	107	3*	97

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[16]

Note 1. In these reactions catalyzed by CK, Mg^{+2} is a required ion in the reaction. However, the ideal range of concentrations required for Mg^{+2} is very narrow and too much of it has an inhibitory effect. In addition, many metal ions such as Mn^{+2}, Ca^{+2}, Zn^{+2}, and Cu^{+2} inhibit CK enzyme activity. Similarly, enzyme activity is inhibited by ADP and citrate, fluoride, nitrate, acetate, iodide, bromide, malonate and L-thyroxine.[18] Urate and cystine are potent inhibitors of the enzyme in serum. Also, chloride and sulfate ions inhibit the activity of the enzyme and the concentrations of these ions must be kept low in any enzyme assay based on the CrP + ADP (reverse) reaction.[1]

Note 2. Macro-CK type 1 has no pathologic significance, but could be the cause of increased CK results in serum, leading to diagnostic confusion and unnecessary further investigations. The prevalence is estimated to be between 0.8 and 2.3%, but this depends on the population studied.[1,19]

Note 3. Macro-CKs may interfere with the CK-MB assay by some immune inhibition methods and may be detected as abnormally displaced bands by electrophoresis. When electrophoretic separation is not possible, the polyethylene glycol (PEG) 6000 precipitation method can be used.[1]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[16]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD**:** For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.

P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests