CK-NAC

En

REF 08V3525

REF 08V3520

Alinity c

Diagnostic reagent for the determination of CK- NAC concentration.
Liquid. Dual reagent. (Ratio: R1/R2: 4/1). Store at +2/+8°C. For In Vitro Diagnostic use. **Do not freeze.**
The products with 08V3525/08V3520 reference numbers provided are compatible for use with Abbott Alinity biochemistry autoanalyzer series.

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

The instructions must be followed carefully. The reliability of the test results cannot be guaranteed unless these instructions are followed.

NAME

CK-NAC

INTENDED USE

CK-NAC assay is used for the quantitative determination of CK-NAC concentration in human serum and plasma on Alinity c analyzer.

SUMMARY AND EXPLANATION OF THE TEST

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]

PRINCIPLES OF THE PROCEDURE

The test has N-Acetyl-L-cysteine (NAC) measurement method. 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.

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

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

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

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]

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]

REAGENTS

Kit Contents

R1 & R2 Imidazole buffer (≤120 mmol/L). Creatine phosphate (≤ 34 mmol/L).D-Glucose (≤ 22 mmol/L). N-Acetylcysteine (≤ 22 mmol/L). Magnesium acetate (≤ 12 mmol/L). EDTA (≤ 2.2 mmol/L). ADP (≤ 2.2 mmol/L). AMP (≤ 5.5 mmol/L). Diadinose pentaphosphate (≤ 15 µmol/L). G6PDH (> 1.5 kU/L). Hexokinase (> 2.5 kU/L). NADP (≤ 2 mmol/L).

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.

Reagent Handling

- Reagent cartridges should be kept in an upright position for at least 1 hours after receipt and before use to eliminate any air bubbles that may have formed inside.
- If the cartridge is dropped, it should be kept upright for 1 hours before use to allow the air bubbles to dissipate.
- Foam and air bubbles may form in the reagents; this may prevent accurate detection of the reagent level inside the cartridge, leading to insufficient reagent aspiration and potentially affecting the analysis results.

Reagent Storage [7]

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	+2/+8°C	Until expiration date	Store in upright position.
Onboard	System Temperature	*60 days*	

Indications of Reagent Deterioration

When a calibration error occurs or the control value falls outside the accepted range, the reagents may be considered to have deteriorated. In this case, the test results are not valid and the samples should be reanalyzed. Recalibration may be required.

Alternative Result Units

Use the unit conversion table provided below for alternative result units.

Default Unit	Conversion Factor	Result Unit
U/L	0.0167	µkat/L

SAMPLE COLLECTION AND PREPARATION FOR ANALYSIS

Sample Types

The recommended sample types for this test are listed below.

Sample Type	Collection Tube
Serum	Serum tubes
Plasma	Li-heparine and sodium heparine sample collection tubes

Follow the instructions provided with your sample collection tubes for use and handling.

Sample Conditions

- To obtain reliable results, serum should be free of fibrin, blood cells, or foreign particles. In samples obtained from individuals receiving anticoagulant or thrombolytic therapy, fibrin residues may be observed due to incomplete clotting.
- To reduce the risk of contamination between samples, a new pipette or pipette tip should be used for each procedure.
- Icteric samples should not be used.
- Samples for CK analysis include serum and heparinized plasma. Since anticoagulants other than heparin inhibit CK activity, they should not be used during blood collection.

Preparation for Analysis

- When preparing sample tubes, the manufacturer's instructions should be followed; separation by standing alone is not sufficient.
- Samples should be free of air bubbles. Before analysis, bubbles should be removed using a stick, and a separate stick should be used for each sample.
- To ensure accurate measurements, the plasma sample tube must be filled to the specified minimum volume to ensure the appropriate anticoagulant-to-sample ratio.[21]
- If the samples contain fibrin, red blood cells, or other particulate matter, centrifuge them again before testing to ensure reliable results.
- If samples do not appear homogeneous, they should be mixed by low-speed vortexing or by inverting the tube 10 times.

Sample Storage

Temperature	Maximum Storage Time
+2/+8°C	2 days
+20/+25°C	8 hours
-20°C	1 months

CK activity in serum is relatively unstable and rapidly declines during storage. The average stability is less than 8 hours at room temperature, 48 hours at 4°C, and 1 month at -20°C. Therefore, if the sample is not analyzed immediately, the serum sample should be refrigerated at 4 °C; if the analysis is delayed for more than 30 days, it should be stored at -80 °C.[1]

The information provided applies to serum and plasma samples. Repeated freeze–thaw cycles of samples should be avoided.

PROCEDURE

Materials Provided
CK-NAC Reagent Kit
Materials Required but Not Provided

- Multiconstituent Calibrator (Lyophilized)
- Commercially available controls
- Saline solution (0.85%–0.90% NaCl) for sample dilution

Assay Procedure

- If primary or aliquot tubes are used, ensure that a sufficient amount of sample is available.
- To prevent evaporation of the specimen, make sure that the tube volume is adequate before each test.
- Minimum Sample Volume Requirements:
  - Sample volume for a single test: 6,9 µL.
  - This volume does not include dead space or the additional aspiration volume.

Sample Dilution Procedures
This method demonstrates measurement linearity up to 2000 U/L. For samples with results above this value, the automatic dilution procedure of the autoanalyzer should be applied or manual dilution may be performed.

Automatic Dilution Protocol
The system dilutes the sample at a 1:10 ratio and automatically calculates the concentration by multiplying the result by the dilution factor.

Manual Dilution Procedures
Dilute the sample with a 0.85%–0.90% NaCl solution. Enter the dilution factor on the relevant screen of the device. If the user does not enter the dilution factor, the result must be manually multiplied by the correct factor before it is reported. If the result of the diluted sample is below 5 U/L, which is the lower limit of the measurement range, the result is not reported, and the procedure is repeated with an appropriate dilution.

Calibration
The use of a Lyophilized Multiconstituent Calibrator is required for this test.

Multiconstituent Calibrator (Lyophilized)
Ref.No: 06R56-02

Calibration stability is 30 days. Changes in the reagent lot number require recalibration. Calibration is necessary if quality control results are not within acceptable limits.

Quality Control Procedures
Commercially available control materials with values assigned by this method may be used.

Traceability is provided with the ERM-AD455k IFCC Creatine Kinase Isoenzyme MM (CK-MM) material.
At least two levels of control should be run every 24 hours. Each laboratory should establish its own quality control procedures. If more stringent control monitoring is required, refer to the quality control procedures established for your laboratory.

EXPECTED VALUES

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

Reference Range [8,20]
Serum / Plasma
Women : 29 - 168 U/L
Men : 30 - 200 U/L

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

			Within-Run (Repeatability)[12,13]		Within-Laboratory (Total)*[12,13]	
Sample	n	Mean (U/L)	SD	%CV	SD	%CV
Level 1	80	153.3	1.68	1.1	5.21	3.4
Level 2	80	278.2	2.22	0.8	8.62	3.1

*: Includes within-run, between-run, and between-day variability.

Lower Measurement Limits
The lower measurement limits refer to the range between the lower limit of quantitation (LLoQ) and the upper limit of quantitation (ULoQ), within which the analyte concentration can be measured with the required medical and laboratory accuracy without dilution, concentration, or any pre-treatment.[9] The LoD and LoQ values have been verified using the CLSI EP17-A2:2012 protocol.[10]

	U/L
LoD**	3.2
LoQ***	5.0

**** :** The LoD represents the lowest concentration at which the analyte can be detected with 95% probability, based on ≥ 60 replicates of samples with low analyte levels.
*****:** When determining the LoQ value, a coefficient of variation (%CV) of ≤20% is used as the basis.

Measuring Interval
This method shows measurement linearity in the activities between 5-2000 U/L. For samples with results above this value, the automatic dilution procedure should be applied. For additional information, please refer to the instrument's user manual. Linearity studies data have been verified by using CLSI EP06-A:2003 protocol.[11]

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.

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

*In calculating how many times the control and test samples (containing interferent), prepared as a serum pool, would be run repetitively, the within-run SD values previously determined for the relevant method and the total allowable error rate defined as the interference limit were used. In the calculations, a type 1 error (α error) rate of 5% and a type 2 error (β error) rate of 10% (90% power) were accepted.[16]

In these reactions catalyzed by CK, Mg^{2+} is an absolutely necessary ion. However, the ideal concentration range for Mg^{2+} is very narrow, and when its concentration is too high, 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 also inhibited by ADP and citrate, fluoride, nitrate, acetate, iodide, bromide, malonate, and L-thyroxine.[18] Urate and cysteine are potent inhibitors of the enzyme in serum. In addition, chloride and sulfate ions inhibit enzyme activity, and the concentrations of these ions must be kept low in any enzyme assay based on the CrP + ADP (reverse) reaction.[1]

Macro-CK type 1 has no pathological significance, but it can be the cause of elevated CK levels in serum, which leads to diagnostic confusion and unnecessary further testing. The prevalence is estimated to range from 0.8% to 2.3%, although this depends on the population studied.[1,19]

Macro-CKs can cause interference in the CK-MB test through certain immunosuppressive methods and may be detected as abnormally displaced bands on electrophoresis. In cases where electrophoretic separation is not possible, the polyethylene glycol (PEG) 6000 precipitation method can be used.[1]

It should be noted that, in addition to endogenous interferents, various drugs and metabolites; anticoagulants (e.g., heparin, EDTA, citrate, oxalate) and preservatives (e.g., sodium fluoride, iodoacetate, hydrochloric acid); substances that may come into contact with samples during collection and handling (such as serum separation devices, sample collection containers and their contents, catheters, catheter flushing solutions, skin disinfectants, hand sanitizers and lotions, glassware detergents, powdered gloves, etc.); dietary substances known to affect certain tests (e.g., caffeine, beta-carotene, poppy seeds); or certain substances present in a sample that may cause interference due to foreign proteins (e.g., heterophilic antibodies), autoimmune responses (e.g., autoantibodies), or malignancy (e.g., paraproteins causing interference in phosphate tests and indirect ion-selective electrode methods) may lead to various interferences and erroneous evaluations for many different reasons.[16]

These performance characteristics were obtained using an autoanalyzer. Results may vary when different equipment or manual procedures are used.

Method Comparison

As a result of the statistical evaluation of method comparison data, the table was constructed according to the Passing–Bablok regression method as follows:
Archem CK-NAC – Roche Cobas Integra 400 Plus

	Units	n	Correlation Coefficient	Intercept	Slpoe	Concentration Range
Serum	U/L	100	0.9999	0.68	0.98	8,1-1746

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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

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