BECKMAN COULTER

CE

AU

# Instructions For Use



# CK (NAC)
# Creatine Kinase (CK NAC)

REF OSR6179 4 x 22 mL R1-1, 4 x 4 mL R1-2, 4 x 6 mL R2
OSR6279 4 x 44 mL R1-1, 4 x 8 mL R1-2, 4 x 13 mL R2

**For *in vitro* diagnostic use only.**

**ANNUAL REVIEW**

| Reviewed by | Date | Reviewed by | Date |
|---|---|---|---|
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## PRINCIPLE

### INTENDED USE

Kinetic UV test for the quantitative determination of creatine kinase, EC 2.7.3.2 (CK), in human serum and plasma on Beckman Coulter AU analysers.

### SUMMARY AND EXPLANATION

Reference[1,2,3,4]

Creatine kinase (CK), a dimer composed of M-muscle and /or B-brain subunits which associate to form the isoenzymes CK-MM, CK-MB and CK-BB, catalyses the reversible phosphorylation of creatine by ATP. Measurements of CK are primarily used in the diagnosis and treatment of myocardial infarction as well as being the most sensitive indicator of muscle damage. CK is increased whenever there is necrosis or regeneration of muscle and is therefore elevated in most myopathies such as Duchenne-muscular dystrophy and in conditions associated with muscle necrosis such as rhabdomyolysis. Total CK can also be increased in diseases of the CNS such as Reyes Syndrome where a 70 fold increase in CK activity indicates the severity of the encephalopathy.

CK-BB predominates in the brain, prostate, gut, lung, kidney, bladder, uterus, liver, thyroid and the placenta. CK-MM predominates in skeletal and cardiac muscle. In healthy individuals the total activity consists mainly of CK-MM while the other CK isoenymes and variants are only present in trace amounts or are undetectable. CK-MB is present to varying degrees in heart muscle and also to a minor degree in skeletal muscle.

CK activity rises following myocardial damage, with a significant increase in both the CK-MM and CK-MB fractions. The proportional rise in the CK-MB fraction to some extent depends on the size of the myocardial damage and on a history of previous myocardial damage. Changes in the ratio of CK-MB to CK-MM may be used to diagnose a myocardial infarct (MI), the ratio reaching a peak within 1.5 hours post MI. The diagnostic sensitivity and specificity of total CK estimation for the diagnosis of an MI can be improved by determining the rate of increase ("slope") of CK on serial samples obtained on admission and at 4, 8 and 12 hours thereafter. A 50% incremental increase per hour over the time period differentiates between an acute MI and non-infarction with an overall efficiency of 94%.

For patients in need of an early diagnosis of a myocardial infarction a rapidly appearing biomarker such as CK-MB plus a biomarker that rises later e.g. cardiac troponin is recommended for confirmation of the diagnosis.

### METHODOLOGY

Reference[5]

Method based on the recommendations of the “International Federation of Clinical Chemistry” (IFCC).

CK reversibly catalyses the transfer of a phosphate group from creatine phosphate to adenosine diphosphate (ADP) to give creatine and adenosine triphosphate (ATP) as products. The ATP formed is used to produce glucose-6-phosphate and ADP from glucose. This reaction is catalysed by hexokinase (HK) which requires magnesium ions for maximum activity. The glucose-6-phosphate is oxidised by the action of the enzyme glucose- 6-phosphate dehydrogenase (G6P-DH) with simultaneous reduction of the coenzyme nicotinamide adenine dinucleotide (NADP) to give NADPH and 6-phosphogluconate. The rate of increase of absorbance at 340/660 nm due to the formation of NADPH is directly proportional to the activity of CK in the sample.

### CHEMICAL REACTION SCHEME

| | | |
|---|---|---|
| Creatine phosphate + ADP | CK | Creatine + ATP |
| ATP + Glucose | HK | ADP + Glucose-6-phosphate |
| Glucose-6-phosphate + $NADP^+$ | G6P-DH | 6-Phosphogluconate + NADPH + $H^+$ |

## SPECIMEN

### TYPE OF SPECIMEN

Serum is the recommended specimen. Haemolysed samples should be avoided. Allow specimen to clot and remove serum from cells promptly to minimise haemolysis and contamination by adenylate kinase from the red cells.

Stable in serum, protected from light, for 8-12 hours when stored at 2…8°C and 4 hours when stored at 15…25°C.[5,6]

Heparinised plasma, free from haemolysis, can also be used. Plasma samples may occasionally produce unpredictable rate reactions resulting in false low results.[5]Plasma with EDTA, oxalate or citrate is not recommended.

## REAGENTS

### WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

### REACTIVE INGREDIENTS

Final concentration of reactive ingredients

| | |
|---|---|
| Immidazole (pH 6.5 @ 37°C) | 100 mmol/L |
| NADP | 2.0 mmol/L |
| ADP | 2.0 mmol/L |
| AMP | 5.0 mmol/L |
| EDTA | 2.0 mmol/L |
| Glucose | 20 mmol/L |
| Creatine phosphate | 30 mmol/L |
| N-acetylcysteine | 0.2 mmol/L |
| Activator | 26 mmol/L |
| $Mg^{2+}$ | 10 mmol/L |
| Diadenosine pentaphosphate | 0.01 mmol/L |
| HK | ≥ 4.0 kU/L |
| G6P-DH | ≥ 2.8 kU/L |
| Stabilisers | |
| Preservative | |

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

**⚠ CAUTION**

**Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).**
**To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.**

## GHS HAZARD CLASSIFICATION

| | | |
|---|---|---|
| CK NAC R1-1 | DANGER | |
| |  | |
| | H316 | Causes mild skin irritation. |
| | H360 | May damage fertility or the unborn child. |
| | P201 | Obtain special instructions before use. |
| | P280 | Wear protective gloves, protective clothing and eye/face protection. |
| | P308+P313 | IF exposed or concerned: Get medical advice/attention. |
| | P332+P313 | If skin irritation occurs: Get medical advice/attention. |
| | | Imidazole 0.5 - < 1% |
| CK NAC R1-2 | DANGER | |
| |  | |
| | H316 | Causes mild skin irritation. |
| | H360 | May damage fertility or the unborn child. |
| | P201 | Obtain special instructions before use. |
| | P280 | Wear protective gloves, protective clothing and eye/face protection. |
| | P308+P313 | IF exposed or concerned: Get medical advice/attention. |
| | P332+P313 | If skin irritation occurs: Get medical advice/attention. |
| | | Imidazole 0.5 - < 1% |
| | | Thioglycerol 1 - 5% |
| CK NAC R2 | WARNING | |
| |  | |
| | H317 | May cause an allergic skin reaction. |
| | H412 | Harmful to aquatic life with long lasting effects. |
| | P273 | Avoid release to the environment. |
| | P280 | Wear protective gloves, protective clothing and eye/face protection. |
| | P333+P313 | If skin irritation or rash occurs: Get medical advice/attention. |
| | P362+P364 | Take off contaminated clothing and wash it before use. |
| | | reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05% |

| | |
|---|---|
| SDS | Safety Data Sheet is available at techdocs.beckmancoulter.com |

### REAGENT PREPARATION

**R1:**

The entire contents of bottle R1-2 must be transferred into the entire volume of R1-1. Mix by gentle inversion before placing on board the instrument.

**R2:**

The reagent is ready for use and can be placed directly on board the instrument.

### STORAGE AND STABILITY

The reagents are stable, unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagents stored on board the instrument are stable for 30 days.

### INDICATIONS OF DETERIORATION

Visible signs of microbial growth, turbidity, precipitate, or any change in reagent colour may indicate degradation and warrant discontinuance of use.

## CALIBRATION

### CALIBRATION INFORMATION

The test is run in MB-mode. To provide a robust approach to generate the analyser specific MB factor, it is recommended that 5 separate calibration events should be used. A fresh vial of calibrator, utilising System Calibrator Cat No. 66300 in the AB calibration mode, should be used for each of these runs. When calculating the mean factor from the separate runs the data should be examined for obvious outliers which should be repeated and replaced. For the AU2700/AU5400 this procedure needs to be performed for each ring. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

The calibrator value is traceable to the IFCC reference method.

Re-establishment of the analyser specific MB factor is recommended when a critical part of the analyser is replaced.

Reagent blank measurement is recommended when changing to a new lot of reagent.

## QUALITY CONTROL

Controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used.

Each laboratory should establish its own control frequency.

Good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed. Values obtained for the controls should fall within specified limits as defined by the user. If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

## TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement.

# CALCULATIONS

The Beckman Coulter analysers automatically compute the creatine kinase activity of each sample.

# REPORTING RESULTS

### REFERENCE INTERVALS

Reference[7]

| | |
|---|---|
| Male | ≤ 171 U/L (2.85 µkat/L) |
| Female | ≤ 145 U/L (2.42 µkat/L) |

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

# PROCEDURAL NOTES

### INTERFERENCES

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

| | |
|---|---|
| Icterus: | Interference less than 3% up to 40 mg/dL or 684 µmol/L bilirubin |
| Haemolysis: | Interference less than 10% up to 5 g/L haemoglobin. |
| Lipemia: | Interference less than 3% up to 1,000 mg/dL Intralipid |

Refer to Young[8] for further information on interfering substances.

# PERFORMANCE CHARACTERISTICS

### PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

### LINEARITY

The test is linear within an enzyme activity range of 10 – 2,000 U/L (0.17 – 33.33 µkat/L).

### SENSITIVITY

The lowest detectable level on an AU640 analyser was estimated at 3 U/L

The lowest detectable level represents the lowest measurable level of CK that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

### METHODS COMPARISON

Patient serum samples were used to compare this CK-Nac OSR6179 assay on the AU640 against another commercially available CK-Nac assay. Results of linear regression analysis were as follows:

| | | | |
|---|---|---|---|
| y = 0.992 x + 0.026 | r = 1.000 | n = 109 | Sample range = 22 - 1,903 U/L |

**PRECISION**

The following data was obtained on an AU2700 using 3 serum pools analysed over 20 days.

| n = 80 | Within-run | | Total | |
|---|---|---|---|---|
| **Mean U/L** | **SD** | **CV%** | **SD** | **CV%** |
| 99 | 2.35 | 2.37 | 4.51 | 4.55 |
| 270 | 2.7 | 1.00 | 8.64 | 3.20 |
| 810 | 5.22 | 0.64 | 26.59 | 3.28 |

# ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

| Test Name | Description |
|---|---|
| CKN1N | CK NAC (Serum) |

**Setting Sheet Footnotes**
# User defined
* Values set for working in U/L. To work in SI units (µkat/L) divide by 60
§ For use in AB mode only, refer to IFU for further instruction.

**REVISION HISTORY**

Revised GHS section

**Preceding version revision history**

Correct error in French Language

# REFERENCES


1. Mayne PD, ed. Clinical chemistry in diagnosis and treatment, 6th ed. London: Arnold,1994:304-310.

2. Thygesen K, Alpert JS et al. Myocardial infarction redefined-A consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. JACC 2000;36:959-969.

3. Stein W. Creatine kinase (total activity). Creatine kinase isoenzymes and variants. In:Thomas L, ed. Clinical laboratory diagnostics. Use and assessment of clinical laboratory results. Frankfurt/Main: TH-Books Verlagsgesellschaft, 1998:71-79.

4. Moss DW, Henderson RA. Clinical enzymology. In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry. Philadelphia:WB Saunders Company, 1999; 657-662.

5. Horder M, Elser R, Gerhardt W, Mathieu M, Sampson E.J. International Federation of Clinical Chemistry, Scientific division committee on enzymes: Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 7. IFCC method for creatine kinase. Appendix A. Eur J Clin Chem Biochem 1991;29(7):435-56.

6. Moss DW, Henderson RA, Kachmar JF. Enzymes. In: Tietz NW, ed. Fundamentals of clinical chemistry. Philadelphia:WB Saunders Company, 1987:376pp.

7. Schumann G, Klauke R. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalised subjects. Clin Chim Acta 2003;327:69-79.

8. Young DS. Effects of Drugs on Clinical Laboratory Tests, AACC, 5th ed. AACC Press, 2000.



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