



## CREATINE KINASE (CK-Nac)

<b><u>OSR6179</u></b>	4 x 22 mL 4 x 4 mL 4 x 6 mL	R1-1 R1-2 R2
<b><u>OSR6279</u></b>	4 x 44 mL 4 x 8 mL 4 x 13 mL	R1-1 R1-2 R2

### Intended Use

System reagent for the quantitative determination of Creatine Kinase (EC 2.7.3.2) in human serum and plasma on Beckman Coulter AU analyzers.

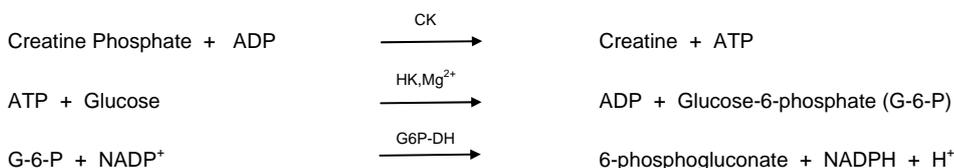
### Summary

Measurements of Creatine Kinase are used in the diagnosis and treatment of myocardial infarction and muscle disease, such as progressive Duchenne-type muscular dystrophy.

Creatine Kinase (CK) is an enzyme which is found primarily in skeletal muscle, cardiac muscle, and brain tissue. Elevated levels of CK are associated with myocardial infarction and various muscle disorders. In myocardial infarction, peak CK levels occur 24 to 36 hours after onset of chest pain and depending on the extent of damage can reach more than 10 times normal levels. In Reye's Syndrome, up to a 70-fold increase in CK activity may be seen due to severe encephalopathy.<sup>3,4</sup>

### Methodology

This CK procedure is a modification of the IFCC method.<sup>1,2</sup> CK reversibly catalyzes 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 catalyzed by hexokinase (HK) which requires magnesium ions for maximum activity. The glucose-6-phosphate is oxidized 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.



### System Information

For AU400/400<sup>®</sup>/480, AU600/640/640<sup>®</sup>/680 and AU2700/5400 Beckman Coulter Analyzers.

### Reagents

Final concentration of reactive ingredients:

Imidazole (pH 6.5)	100 mmol/L	
HK (Yeast)	≥ 4.0 kU/L	(66.7 μkat/L)
NADP	2 mmol/L	
G6P-DH ( <i>Leuconostoc mesenteroides</i> )	≥ 2.8 kU/L	(46.7 μkat/L)
ADP	2 mmol/L	
Mg <sup>2+</sup>	20 mmol/L	
AMP	5 mmol/L	
Diadenosine pentaphosphate	10 μmol/L	
EDTA	2 mmol/L	
Glucose	20 mmol/L	
Creatine Phosphate	30 mmol/L	
N-Acetylcysteine	0.2 mmol/L	
Stabilizers		

Also contains preservatives

### Precautions

1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Contains sodium azide as a preservative, which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

### Preparation of Reagents

R1: Ensure complete transfer of R1-2 into R1-1 by pouring an aliquot of R1-1 buffer into R1-2, mix gently, then transfer entire contents back into 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. No preparation is required.

# Creatine Kinase (CK-Nac)

## Storage and Stability

1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.

## Indications of Deterioration

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

## Specimen Collection and Preparation

Serum and heparinized plasma samples free from hemolysis are the recommended specimens. Allow specimen to clot. Remove serum from cells promptly to minimize hemolysis and contamination by adenylate kinase from the red cells. Plasma samples may occasionally produce unpredictable rate reactions resulting in false low results.<sup>1</sup> Plasma with EDTA, oxalate or citrate is not recommended.

## Sample Storage and Stability

Protect samples from light for maximum stability. CK is stable in serum for 4 hours at 15 - 25°C, 8 - 12 hours at 2 - 8°C or 1 month at ≤ -20°C.<sup>3</sup>

## Interfering Substances

Results of studies<sup>5</sup> show that the following substances interfere with this creatine kinase assay.

The criteria for no significant interference is recovery within 10% of the initial value.

Bilirubin: No significant interference up to 40 mg/dL Bilirubin  
Hemolysis: No significant interference up to 500 mg/dL Hemolysate  
Lipemia: No significant interference up to 1000 mg/dL Intralipid\*

\* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young<sup>6</sup> for a compilation of reported interferences with this test.

## Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

## Materials Provided

CK Reagent

## Stability of Final Reaction Mixture

The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

## Calibration

Calibration of this creatine kinase procedure is based upon the theoretical extinction coefficient for NADPH, which has a molar absorptivity of 6300 at 340/660 nm.

## Quality Control

During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed with each new lot of reagent, after specific maintenance, or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

## Results

Automatically printed out for each sample in U/L at 37°C.

## Dynamic Range

The CK procedure is linear from 10 to 2000 U/L. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated, and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

## Expected Values

Adults:<sup>7</sup> 30 - 223 U/L

Expected values may vary with age, sex, diet, and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

## Specific Performance Characteristics

The following data was obtained using the CK Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

## Precision<sup>9</sup>

Estimates of precision, based on CLSI recommendations<sup>8</sup>, are consistent with typical performance. The within run precision is less than 5% CV and total precision is less than 10% CV. Assays of control sera were carried out and data reduced following CLSI guidelines above.

N= 80 Mean (U/L)	Within run		Total	
	SD	CV%	SD	CV%
117	1.22	1.00	1.77	1.50
135	2.04	1.50	3.83	2.80
452	4.85	1.10	11.47	2.50
498	5.44	1.10	9.94	2.00

## Creatine Kinase (CK-Nac)

### Method Comparison<sup>9</sup>

Patient samples were used to compare this CK Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640 <sup>9</sup>
X Method	Method 2
Slope	0.997
Intercept	0.392
Correlation Coeff. (r)	0.9997
No. of Samples (n)	103
Range (U/L)	20 - 584

### Sensitivity

Typical change in absorbance per minute for 1 U/L of CK is 0.12 mAbsorbance.

### References

1. Horder M., Elsner R., et al., Approved Recommendation of IFCC Methods for the Measurement of Catalytic Concentration of Enzymes, Part 7 IFCC Method for Creatine Kinase. J. Clin. Chem. Clin. Biochem. 29, 435, 1991.
2. Szasz, G., Gerhardt, W. and Gruber, W., Clin Chem, 23: 1888, 1977.
3. Tietz, N.W. (ed), Fundamentals of Clinical Chemistry, 3<sup>rd</sup> Edition, W.B. Saunders, 1987.
4. Tietz, N.W., (ed), Clinical Guide to Laboratory Tests, 3<sup>rd</sup> Edition, W.B. Saunders, 1995.
5. CLSI/NCCLS, Interference Testing in Clinical Chemistry EP7-P, 1986.
6. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5<sup>th</sup> Edition, AACC Press, 2000.
7. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
8. CLSI/NCCLS Evaluation Protocol, EP5-A, 1999.
9. Data is on file for specific AU analyzers.

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