CK NAC (IFCC)

INTENDED USE
Bioline CKNAC for the quantitative determination of Creatine Kinase in human serum.

CLINICAL SIGNIFICANCE
Creatine Kinase (CK) plays an important role in the energy-storing mechanism of tissue by catalyzing the reversible reaction between creatine and ATP to form creatine phosphate and ADP.CK is distributed in various organs; the highest activities (in decreasing order) are skeletal muscle, heart, and brain.[1]Thus, determination of CK is an aid in diagnosing muscular dystrophy and other diseases of the skeletal muscles, myocardial infarction, hypothyroidism, renal diseases ,and/or dysfunction.[2]

METHOD AND PRINCIPLE
The early procedure for determining CK was based on the rate of ATP formation.[3]A modified method was described by Nielson by adding a sulfhydryl compound and AMP to assure maximum CK activity and inhibit adenylate kinase activity.[4]Optimizedconditions for measuring CK were published by Szasz in 1976 as well as by the Scandinavian committee on enzyme. The above procedure was modified again in1979 to include EDTA. The present reagent is a modification of the above revision.

Reaction Sequence:

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

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

$$\text{Glucose-6-phosphate} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{6-Phosphogluconate} + \text{NADH} + \text{H}^{+}$$

CK catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. The ATP and glucose are converted to ADP andglucose-6-phosphate by hexokinase(HK) .Glucose-6-phosphate dehydrogenase (G-6-PDH) oxidizes at the D-glucose-6-phosphate and reduces the nicotinamide adenine dinucleotide (NAD).The rate of NADH formation, measured at 340nm, is directly proportional to serum CK activity.

REAGENTCOMPOSITION
When reconstituted as directed, the reagent for CK contains the following:

D-Glucose	20mM
Magnesium^{++}	10mM
Adenosine-5'-Monophosphate(AMP)	50mM
N-Acetylcysteine (NAC)	20mM
Creatine Phosphate	30mM
Adenosine-5'-Diphosphate(ADP)	2mM
OxidizedNicotinamide Adenine DinucleotidePhosphate	2mM
Glucose-6-Phosphate Dehydrogenase (E.C.I.I.I.49,G-6-PDH)	3,000UL
Hexokinase(E.C.2.7.1.1, HK)	3,000UL
EDTA	2mM
Buffer	100mM

WARNINGS AND PRECAUTIONS
1. For *in vitro* diagnostic use.
2. Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

REAGENT PREPARATION
Reagent R1 and R2 are ready to use, To prepare working reagent mix R1 and R2 in the ratio of 4:1.

STORAGE AND STABILITY OF REAGENT.
The reagent should be stored at 2-8°C.The reagent may be used until the expiration date indicated on the package label. After reconstitution, the reagent is stable for twenty-four(24)hours at room temperature or14 days refrigerated (2-8degC).

REAGENT DETERIORATION
1. Physical Appearance
   If reagent appears turbid deterioration may have occurred and the product should be discarded.
2. Blank Absorbance
   If the reconstituted CK REAGENT without added sample has an absorbance greater than 0.700 at 340 nm versus reagent grade water, the reagent is considered to be unsatisfactory for use and should be discarded.
3. Control Assays
   Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration.
4. We cannot guarantee the stability of reagents which have been:
   a. Transferred from their original containers.
   b. Improperly stored prior to or during use
   c. Contaminated during use

SPECIMENCOLLECTION AND STABILITY
Collect whole blood by non-traumatic venipuncture and allow to clot. Centrifuge and remove serum immediately. Serum is reportedly stable for four (4) hours at room temperature, 8-12 hours at 4°C, and 2-3 days when frozen.
Hemolyzed specimens should not be used because of side reactions that may occur due to adenylate kinase, adenosine triphosphate, and glucose-6-phosphate dehydrogenase liberated from red cells.

INTERFERENCES
Certain drugs and medications may affect the activity of CK, see Young et al.[9]

MANUAL PROCEDURE
Wavelength 340nm
Temperature 37ºC

	Test
ReagentR1	800µL
ReagentR2	200µL
Sample	40µL

Mix and after 3 min of incubation measure the optical density during next 90 sec.

1. Prepare working reagent according to instructions.
2. Pipette 1.0ml of reagent into appropriate tubes and pre-warm at37ºC.
3. Zero spectrophotometer with distilled water at 340nm.
4. Add 0.040ml (40ul)of sample to the reagent ,mix, and incubateat 37ºC for 3 minutes.
5. After 3 minutes, read and record the absorbance. Return the tubes to 37ºC.Repeat readings every 30sec sec for the next 90 sec.
6. Calculate the average absorbance difference per minute(ΔAbs./min.)
7. The Abs./min. multiplied by the factor 4127(seeCalculations) will yield results in IU/L.
8. Samples with values above 2000 IU/L should be diluted 1:1with saline, re-assayed, and the results multiplied by two (2).

CALCULATIONS

IU/L = ΔA Abs./min.x4127

LIMITATIONS

1. Some inhibitorsofCKactivity[10]
   a. Excessive Mg^{++},Cl^{-},SO^{2-}_{4}
   b. Most heavy earth metals,i.e.Zn^{++},Cu^{++}, Mn^{++}
   c. Iodoacetate, and other sulfhydryl binding agents
   d. Excess ADP, citrate, fluoride, L-thyroxine
   e. Excess uric acid
2. This procedure measures total CK activity irrespective of its tissue or organ of origin.
3. Lower than expected CK values have been reported in samples having high alkaline phosphatase activity.

QUALITYCONTROL

Use control sera with known normal and abnormal values to monitor the integrity of the reaction. Values should be those acceptable for this method and temperature.

EXPECTEDVALUES

25- 192 IU/L(37°C)
10- 109 IU/L(30°C)
It is strongly recommended that each laboratory establish it own normal range.

PERFORMANCE CHARACTERSTICS

1. Linearity:2000 IU/L
2. Comparison: Studies done between this procedure and a similar procedure yield a correlation coefficient of 0.991 with a regression equation of Y = 1.01X - 0.29.
3. Precisionstudies:

Mean(mg/dl)	S.D.	Within Run	C.V.%
111	1.6		1.5
373.5	12.4		3.3
Mean(mg/dl)	**S.D.**	**RuntoRun**	**C.V.%**
110.9	4.3		3.9
367.4	10.3		2.8

Mode	**Kinetic**
Wavelength (Filter)	**340nm**
Reaction Direction	**Increasing**
Sample Vol.	**40µl**
Reagent Vol.	**1000 µl**
Delay Time / Lag Time	**180 Sec**
Measuring Time	**90 Sec**
Reagent Blank Abs.(Max)	**NMT 0.700**
Calibration Method	**Fix factor**
Factor	**4127**
Linearity	**2000 IU/L**
Decimal Places	**1**
Temp.	**37ºC**
Unit	**IU/L**
Ref. Low (Male / Female)	**25 IU/L**
Ref. High (Male / Female)	**192 IU/L**

REFERENCES

1. Faulker, W.R. and Meites, S.:*Selected Method of ClinicalChemistry.* vol 9, p. 185 (1982).
2. Rosalki, S.B.: *J. Lab. Clin. Med.*69:696 (1967).
3. Oliver, I.T.: *Biochem. J.* 61:116 (1955).