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CREATINE KINASE (CK-NAC) LIQUID REAGENT (UV-KINETIC METHOD)

INTENDED USE
For the quantitative determination of Creatine Kinase in human serum.

INTRODUCTION
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]

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] Optimized conditions for measuring CK were published by Szasz in 1976 as well as by the Scandinavian committee on enzyme.[5,6] The above procedure was modified again in 1979 to include EDTA.[7] The present reagent is a modification of the above revision.

PRINCIPLE
Reaction Sequence:

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

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

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

CK catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. The ATP and glucose are converted to ADP and glucose-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.

REAGENT COMPOSITION
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
Oxidized Nicotinamide Adenine Dinucleotide Phosphate	2mM
Glucose-6-Phosphate Dehydrogenase (E.C.l.l.l.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
The working reagent is prepared by mixing five (5) volumes of R1 with one (1) volume of R2 in a disposable container.
Example: 25 ml R1 + 5 ml R2

STORAGE AND STABILITY
The reagent should be stored at 2-8°C prior to reconstitution. 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 or twenty-one (21) days refrigerated (2-8°C).

REAGENT DETERIORATION
1. Physical Appearance
   If reagent appears damp and clumped, 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 0.70 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

SPECIMEN COLLECTION
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.[8]

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.

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

MATERIALS REQUIRED BUT NOT PROVIDED
Sample and reagent pipettes, test vials or cuvettes, timer, test tube rack, thermoregulated flowcell, spectrophotometer, control serum.

GENERAL INSTRUCTIONS
The reagent for CK is intended for use either as automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

PROCEDURE (AUTOMATED)
See appropriate instrument application instructions.

PROCEDURE (MANUAL)

1. Prepare the working reagent according to instructions.
2. Pipette 1.0ml of reagent into appropriate tubes and pre-warm at 37°C for four (4) minutes.
3. Zero spectrophotometer with distilled water at 340nm.
4. Add 0.025 ml (25µl) of sample to the reagent, mix, and incubate at 37°C for two (2) minutes.
5. After two minutes, read and record the absorbance. Return the tubes to 37°C. Repeat readings every minute for the next two minutes.
6. Calculate the average absorbance difference per minute (Δ Abs./min.)
7. The Δ Abs./min. multiplied by the factor 6592 (see Calculations) will yield results in IU/L.
8. Samples with values above 1,200 IU/L should be diluted 1:1 with saline, re-assayed, and the results multiplied by two (2).

NOTE: If the spectrophotometer being used requires a final volume greater than l.0ml for accurate readings, 3ml of reagent, and 0.1ml (100µL) of sample may be used.
If the spectrophotometer being used is equipped with a temperature-controlled cuvette, the reaction mixture may be left in the cuvette while the absorbance readings are taken.

PROCEDURE LIMITATIONS

1. Some inhibitors of CK activity[10]
   a. Excessive Mg^{++}, Cl^{-}, SO_4^{2-}
   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.

CALCULATIONS

$$\text{IU/L} = \frac{\Delta\text{Abs./min.} \times \text{TV} \times 1000}{\text{d} \times \in \times \text{SV}} = \frac{\Delta\text{Abs./min.} \times 1.025 \times 1000}{1 \times 6.22 \times 0.025}$$

$$= \text{A Abs./min.} \times 6592^{*}$$

Where:
- ΔAbs./min. = Average absorbance change per minute
- TV = Total reaction volume (1.025)
- 1000 = Conversion of IU/ml to IU/L
- d = Light path in cm (1.0)
- ∈ = Millimolar absorptivity of NADH (6.22)
- SV = Sample volume in ml (0.025)

Example: If your average absorbance change per minute is 0.015, then 0.015 x 6592 = 98.9 IU/L.

NOTE: If any of the test parameters are changed, a new factor has to be determined using the above formula.

SI UNITS: To convert to SI Units (nKat/L) multiply IU/L by 16.67.

* If 3ml of reagent and 0.1ml (l00ul) of samples are used, then IU/L = Δ Abs/min. x 4984.

QUALITY CONTROL

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.

TEMPERATURE CONVERSION FACTORS

You may convert results to approximate results at other temperatures by multiplying by the appropriate temperature factor.

Performance Temperature	Reporting Temperature		
	25°C (TF)	30°C (TF)	37°C (TF)
25°C	1.00	1.38	2.30
30°C	0.72	1.00	1.67
37°C	0.43	0.60	1.00

EXPECTED VALUES

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

PERFORMANCE

1. Linearity: 1,200 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. Precision studies:

Within Run

Mean (mg/dl)	S.D.	C.V.%
111	1.6	1.5
373.5	12.4	3.3

Run to Run

Mean (mg/dl)	S.D.	C.V.%
110.9	4.3	3.9
367.4	10.3	2.8

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