CK-NAC (UV-Rate)
Procedure No. FT682

For the quantitative determination of Creatine Kinase in serum or plasma

Summary and Principle

Serum creatine kinase (CK) levels have proven valuable in the assessment of cardiac and skeletal muscle diseases, including myocardial infarction and muscular dystrophy[1]. Determination of creatine kinase and lactate dehydrogenase isoenzymes provides a definitive diagnosis of acute myocardial infarction.[2]

The kinetic procedure presented is a modification of Szasz[3] of the Rosalki[4] technique, which optimizes the reaction by reactivation of CK activity with N-acetyl-L-cysteine (NAC).

CK specifically catalyzes the transphosphorylation of ADP to ATP. Through a series of coupled enzymatic reactions, NADH is produced at a rate directly proportional to the CK activity. The method determines NADH absorbance increase per minute at 340 nm.

Reagents

CK Reagent (powder), Cat. No. FT682a

Contents:

ADP	2.0 mmol/L
AMP	5.0 mmol/L
Diadenosinepentaphosphate	10 mmol/L
NAD	2.0 mmol/L
HK	3000 U/mL
G6P-DH	3000 U/mL
N-acetylcysteine	20 mmol/L
Creatine phosphate	30 mmol/L
D-glucose	20 mmol/L
Magnesium ++	10 mmol/L
EDTA	2 mmol/L
Buffer	100 mmol/L

Precautions: For In Vitro Diagnostic Use.

Reagent Preparation: Add volume of deionized water to each vial as stated on label. Swirl gently to dissolve contents.

Reagent Storage and Stability: The Reagents are stable until expiration date on label. Reconstituted reagent stable 24 hours at room temperature (15-30°C) or 30 days at 2-8°C. Dry reagent should have a uniform white to off-white appearance. If the reagent blank absorbance without added serum exceeds 0.700 at 340 nm, and 1 cm light path, reagent may have deteriorated and should not be used.

Materials Required But Not Provided

Spectrophotometer capable of absorbance readings at 340 nm and 2 cm lightpath
Constant temperature block or bath, 30°C or 37°C, or temperature controlled cuvet well
Accurate pipetting devices
Test tubes
Vortex mixer
Interval timer

Specimen Collection and Preparation

1. Clear unhemolyzed serum is the recommended sample. No special additives or preservatives are required. In addition, heparinized plasma may be used.
2. Intramuscular injections or strenuous physical exercise may elevate serum CK.

Sample Stability: Store serum in stoppered tubes. CK activity in serum is reportedly stable for three days at 2-8°C. Addition of sulfhydryl agents preserves CK activity during prolonged storage.[5, 6] Some control sera, however, show a considerable decrease in CK activity only a few hours after reconstitution.

Interfering Substances: Chloride and sulfate inhibit CK activity. Young et al.[7] have reviewed drug effects on serum CK levels.

Automated Analyzers

Parameters:

Wavelength	340 nm
Reaction Type	Kinetic
Reaction Direction	.Increasing
Reaction Temperature	37°C
Sample/Reagent Ratios	1:40
Equilibration Time	2 minutes
Blank Absorbance Limit	0.700A
High Absorbance Change/Min	0.250 Δ A/Min.
Factor	.6592
Low Normal	25 U/L (37°C)
High Normal	192 U/L (37°C)
Linearity	1500 U/L
Cuvet Lightpath	1 cm

Above parameters should be employed in programming automated analyzers for CK. Consult your instrument manual for programming instructions.

Manual Procedure

1. For each sample add 1.0 mL reconstituted CK Reagent into a cuvet or test tube and warm to 37°C for approximately 5 minutes.
2. Add 0.025 mL (25 µL) sample to its respective tube, mix well and incubate 2 minutes at 37°C.
3. Set the wavelength of the instrument at 340 nm. Zero with deionized water.
4. Record the increase in absorbance at 60 second intervals (Δ A/Minute) for 3 minutes. The rate of change should be constant.

NOTE: If cuvet is not temperature controlled, incubate samples at 37°C between readings.

Quality Control: Two levels of control material with known CK levels determined by the model should be analyzed each day of testing.

Results

Values are derived based on the "absorptivity micromolar extinction coefficient" of NADH at 340 nm (0.00622). A unit per liter (U/L) of CK activity is that amount of enzyme which oxidizes one µmol/L of NADH per minute.

$$U/L = \frac{\Delta\ A/Min}{Absorptivity} \times \frac{Total\ Volume}{Sample\ Volume}$$

$$U/L = \frac{\Delta\ A/Min}{0.00622} \times \frac{1.025}{0.025}$$

$$U/L = \Delta\ A/Min \times 6592$$

Expected Values

10-109 U/L (30°C)
25-192 U/L (37°C)

The range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

Performance Characteristics

Reproducibility: Studies were accomplished (within day) by 20 assays on each of 2 control sera. These samples were again assayed daily for 10 days (day-to-day).

Within Day:	**Mean**	**SD**	**CV %**
Normal	111.1	1.6	1.5
Abnormal	373.5	12.4	3.3
Day to Day:	**Mean**	**SD**	**CV %**
Normal	110.9	4.3	3.9
Abnormal	367.4	10.3	2.8

Linearity: To 1500 U/L, samples exceeding this value should be diluted 2-fold (1 + 1) with deionized water, the assay repeated and results multiplied by the dilution factor of 2.

References

1. Kachmar JF, Moss DW. In Fundamentals of Clinical Chemistry, 2nd ed. NW Tietz, Editor. WB Saunders, Philadelphia, 1976, p 682.
2. Row CR et al. J Lab Clin Med 80:557, 1972.
3. Szasz G. Proceedings of the Second International Symposium on Clinical Enzymology, Chicago, October 1975.
4. Rosalki SB. J Lab Clin Chem 23:646, 1977.
5. Morin LG, Clin Chem 23:646, 1977.
6. Nealon DA, Henderson AR. Clin Chem 23:646, 1977.
7. Young DS et al. Clin Chem 21:286D, 1975 (Special Issue).

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