

Intended Use

For the quantitative determination of creatine kinase activity in serum.

Summary and Principle

Serum creatinine 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 Szasz³ of the Rosalki⁴ 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, NADPH is produced at a rate directly proportional to the CK activity. The method determines the NADPH absorbance increase per min at 340 nm.

Reagents

CK R1 (buffer) contains: Imidazole buffer (pH 6.7) 100.0 mmol/L; NADP 2.0 mmol/L; HK (Baker's yeast) 2.5 KU/L; Glucose 20.0 mmol/L; Magnesium Acetate 10.0 mmol/L; EDTA 2.0 mmol/L and N-acetylcysteine (NAC) 20.0 mmol/L.

CK R2 (enzyme reagent) contains: Imidazole buffer (pH 6.7) 100.0 mmol/L; ADP 2.0 mmol/L; AMP 5.0 mmol/L; Diadenosine pentaphosphate 10.0 mmol/L; Creatine phosphate 30.0 mmol/L; G₆PDH (Baker's yeast) 1.5 KU/L and EDTA 2.0 mmol/L.

Reagent Preparation

Reagents are supplied as ready to use liquids. To prepare working reagent, mix 4 parts of R1 (buffer) with 1 part R2 (enzyme).

Reagent Storage

1. Reagents should appear clear and colorless. Discard if either appears cloudy or contains particulate matter.
2. Store R1 and R2 at 2-8°C, protected from light. If stored as directed the reagents are stable until the expiration date.
3. Working reagent is stable for 3 weeks at 2-8°C or 2 days at room temperature (15-30°C).

Precautions

1. This reagent is for *in vitro* diagnostic use only.
2. Normal precautions in handling laboratory reagents should be followed.
3. The reagents contain sodium azide which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

Specimen Collection and Handling

1. Clear unhemolyzed serum is the specimen of choice. No special additives or preservatives are required.
2. Whenever possible, specimens should be separated and analyzed on the day of collections and stored in capped tubes.
3. 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.

Interferences

1. Intramuscular injections and strenuous physical exercise may elevate serum CK.
2. Chloride and sulfate inhibit CK activity.
3. Bilirubin levels up to 40 mg/dl and triglyceride levels up to 2000 mg/dl show no interference in this test.⁹
4. Young, et al. have reviewed drug effects on serum CK levels.⁷

Materials Provided

CK R1 and R2 Reagent.

Materials Required but not Provided

1. Test tubes/rack
2. Pipetting devices
3. Spectrophotometer with the ability to read at 340nm and 1 cm light path.
4. Timer
5. Heating Block (37°C)

Procedure (Automated)

Refer to specific instrument application instructions.

Procedure (Manual)

1. Prepare working reagent according to instructions.
2. Pipette 1.0ml of working reagent into appropriate tubes and pre-warm at 37°C for 4 minutes.
3. Zero spectrophotometer with distilled water at 340nm.
4. Add 0.05 ml (50 ul) sample to its respective test tube and mix gently.
5. After two minutes, read and record the absorbance. Return tube to 37°C. Repeat readings every minute for the next two minutes. The rate should be constant.
6. Calculate the average absorbance difference per minute $\Delta\text{Abs./min}$.
7. Multiply the $\Delta\text{Abs./min}$ by the factor 3376 for results in U/L.

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

Calibration

CK activity is based on the "micromolar extinction coefficient" of NADP at 340 nm (see "Calculations" section). The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer. Assaying the CK contents of a control serum with known CK values can be used to assure instrument calibration has been performed correctly.

Calculations

Values are derived based on the "absorptivity micromolar extinction coefficient" of NADP at 340 nm (0.00622). A unit per liter (U/L) of CK activity is that amount of enzyme which oxidizes one $\mu\text{mol/L}$ of NADP per minute.

$$\text{U/L} = \frac{\Delta\text{A/Min}}{0.00622} \times \frac{1.05}{0.05}$$

$$\text{U/L} = \frac{\Delta\text{A/Min}}{0.00622} \times \frac{\text{Total Volume}}{\text{Sample Volume}}$$

$$\text{U/L} = \Delta\text{A/Min} \times 3376$$

Liquid Creatine Kinase Reagent Set

Limitations

If the $\Delta\text{Abs./min}$ is greater than 0.345, dilute 1 part sample with 9 parts saline and re-assay. Multiply results by 10. CK values for neonatal patients have not been established with this procedure.

Quality Control

The validity of the reaction should be monitored by use of control sera with known normal and abnormal creatine kinase values. These conditions should be run at least with every working shift in which creatine kinase assays are performed. It is recommended that each laboratory establish its own frequency of control determination.

Expected Values⁸

Normal range: Males: 38-174 U/L (37°C)
Females: 26-140 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 Characteristics⁹

Comparison: A group of 77 sera ranging in CK activity from 3 - 700 U/L was assayed by the described CK method and by a similar commercially available CK reagent. Comparison of the results yielded a correlation coefficient of 0.999 and the regression equation was $y = 1.027x - 0.65$. (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T).

Precision: Within-run precision was established by 20 assays on three different levels of commercial controls. Total Precision values were obtained by assaying 3 commercial controls for 5 consecutive days.

<u>Within-Run</u>	<u>Serum 1</u>	<u>Serum 2</u>	<u>Serum 3</u>
Mean CK (U/L)	159	220	508
Std. Deviation (U/L)	3.2	1.5	3.7
C.V. (%)	2.0	0.7	0.7

<u>Total Precision</u>	<u>Serum 1</u>	<u>Serum 2</u>	<u>Serum 3</u>
Mean CK (U/L)	50	157	228
Std. Deviation (U/L)	1.1	1.6	2.3
C.V. (%)	2.1	1.0	1.0

Precision studies were performed according to NCCLS Tentative Guideline, EP5-T.

Linearity: Linear from 1 to 1200 U/L at 37°C.⁸ Performed according to NCCLS Guideline EP6-P.

Sensitivity: Based on an instrument resolution of $A = 0.001$, the method presented shows a sensitivity of 1.0 U/L.










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 S.B., 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).

8. Tietz, Norbert W., Clinical Guide To Laboratory Tests, W.B. Saunders Company, Philadelphia, PA., (1995), p180.

9. Manufacturer's Laboratory Data

Symbol Key



 Use by (YYYY-MM)	 Lot and batch code
 Catalog number	 Manufacturer
 In vitro diagnostic medical device	 Temperature limitation
 Consult instructions for use	 CE mark
 Authorized representative in the European Community	

 C7522



 2°C - 8°C



	Manufactured for Pointe Scientific, Inc. 5449 Research Drive, Canton, MI 48188
	Obelis s.a. Boulevard Général Wahis 53 1030 Brussels, BELGIUM Tel: (32)2.732.59.54, Fax:(32)2.732.60.03 email: mail@obelis.net
	

Rev. 3/15 P803-C7522-01