

# Creatine Kinase (CK)

## 1.0 INTENDED USE

This reagent is intended for the quantitative determination of creatine kinase (CK) in serum.

## 2.0 BACKGROUND

### 2.1 METHOD AND HISTORY

The method of assaying creatine kinase (CK) using creatine phosphate and adenosine diphosphate (ADP) as substrates instead of creatine and adenosine triphosphate (ATP) was described by Oliver, Gerhart and Rothauwe (10.1,10.2,10.3). In this procedure N-acetylcysteine (NAC) is the thiol activator and adenosine monophosphate (AMP) and p<sup>1</sup>, p<sup>5</sup>-di(adenosine-5') pentaphosphate (AP<sub>5</sub>A) are added to inhibit the interference caused by adenylate kinase activity.

### 2.2 TEST PRINCIPLE

CK  
Creatinine phosphate + ADP ----> creatine + ATP

HK  
ATP + D-glucose ----> ADP + D-glucose-6-phosphate

G-6-PDH  
D-glucose-6-phosphate + NADP<sup>+</sup> ----->  
D-glucono-d-lactone-6-phosphate + NADPH + H<sup>+</sup>

The rate of increase in absorbance at 340 nm due to the formation of NAD is directly proportional to the creatine kinase activity.

### 2.3 CLINICAL SIGNIFICANCE (10.5)

Creatinine Kinase (CK) is a dimeric molecule composed of two distinct subunits. At least three isoenzymes have been identified. CK is found primarily in heart and skeletal muscle and also in brain tissue. Following a myocardial infarction, CK rises measurably within a 4-6 hour period. Maximal values are observed within 24 hours, after which time, the activity returns to normal. Since CK is present in skeletal muscle, elevations will be observed in muscular dystrophy (particularly the Duchenne type), polymyositis and similar muscle diseases. Any trauma to muscle from bruises, fractures, surgical procedures, frequent intramuscular injections, muscle spasms and even strenuous physical exercise may induce a significant rise in CK activity. Acute cerebrovascular disease with cerebral ischemia will also increase CK values.

## 3.0 SPECIMEN COLLECTION AND HANDLING

### 3.1 PATIENT PREPARATION

No special patient preparation is required.

### 3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolysed serum is the preferred specimen. Use a standard venipuncture tube to draw patient sample. The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25 µl. Call Biotron's technical service department at 1-800-595 8766 for the recommended sample volume for your analyzer. Record the patient's name, date and time of sample collection and preparation.

### 3.3 SPECIMEN STORAGE

In serum, CK is stable for 4 hours at room temperature (18°-26°C), 8 to 12 hours when refrigerated (2°-8°C) and for only 2 to 3 days when frozen (-20°C). (10.6,10.8) It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

## 4.0 MATERIALS (10 X 10 ml) (6 X 50 ml)

Reagents necessary for the determination of creatinine kinase are included in the kit.

### 4.1 REAGENT

CK Reagent contains, after reconstitution with deionized water:

creatine phosphate disodium	≥ 32 mmol/L
ADP	≥ 2.0 mmol/L
glucose	≥ 20 mmol/L
NAD	≥ 2.2 mmol/L
hexokinase	≥ 3,000 U/L
glucose-6-phosphate dehydrogenase	≥ 2,500 U/L

magnesium acetate	≥ 12 mmol/L
EDTA	≥ 2 mmol/L
NAC	≥ 20 mmol/L

## 4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

## 4.3 REAGENT PREPARATION

### 4.3.1

Add 10 ml of deionized water to each of the required number of CK reagent vials. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

### 4.3.2

Add 50 ml of deionized water to each of the required number of CK reagent vials. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

## 4.4 REAGENT STORAGE AND STABILITY

All reagents included in the kit are stable at 2-8° C (refrigerated) until the expiration date stated on the labels. The CK working reagent is stable when refrigerated (2-8° C) for 14 days.

## 4.5 ADDITIONAL MATERIALS REQUIRED

- 4.5.1 Spectrophotometer or colorimeter capable of reading absorbance accurately at 340 nm.
- 4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 340 nm.
- 4.5.3 Test tubes capable of holding 2 ml.
- 4.5.4 Pipettes capable of delivering 1 ml and 25 µl
- 4.5.5 A timer with one minute increments.
- 4.5.6 A water bath which can be adjusted to 37° C.
- 4.5.7 Normal and abnormal control for quality control.

## 5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

### 5.1 PROCEDURE CONDITIONS

Wavelength	340 nm
Temperature	37° C
Pathlength	1 cm
Mode	Kinetic
Reaction time	3 - 5 minutes
Sample volume	25 µl
Reagent volume	1.0 ml
Total volume	1.025 ml
Sample to reagent ratio	1:40

### 5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 340 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

### 5.3 CALIBRATION

No reagent calibration is necessary as the CK activity is calculated by use of the molar absorptivity of NAD, which is taken as 6.22 at 340 nm.

### 5.4 PROCEDURE

5.4.1 Prepare the required volume of CK working reagent. (See 4.3 Reagent Preparation section.)

5.4.2 Into separate test tubes pipette 25 µl of serum to be assayed.

5.4.3 Add 1.0 ml of CK working reagent to each test tube. Mix, and incubate for 3 to 5 three minutes at 37° C. The lag time will be decreased if the reagent is prewarmed to the incubation temperature.

5.4.4 Record the change in absorbance at 340 nm at one minute intervals until the absorbance change is constant.

### 5.5 CALCULATION AND RESULTS

$$CK (U/L) = \frac{\Delta A/\text{min} \times \text{assay volume (ml)} \times 1000}{6.22 \times \text{light path (cm)} \times \text{sample volume (ml)}} = \Delta A/\text{min} \times 6592$$

ΔA/min = change in absorbance per minute

Assay volume = total reaction volume expressed in ml

1000 = converts U/ml to U/L

6.22 = absorbance coefficient of NAD

Lightpath= length of the light path expressed in cm (usually 1.0)

Sample volume = sample volume expressed in ml

6592 = factor derived from the constants in the equation

Example:

$$0.013 \times 1.025 \times 1000$$

$$CK (U/L) = \frac{0.013 \times 1.025 \times 1000}{6.22 \times 1 \times 0.025} = 0.013 \times 6592 = 86 U/L$$

ΔA/min = 0.013

Assay volume = 1.025

Light path in cm = 1

Sample volume in ml = 0.025

### 6.0 INTERPRETATION OF RESULTS

#### 6.1 EXPECTED VALUES (10.3)

The range of expected values for the CK is:

Male: 20-184 U/L (37° C)

Female: 20-160 U/L (37° C)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

#### 6.2 MEDICAL ALERT VALUES (10.9)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

#### 6.3 LIMITATIONS OF PROCEDURE

A comprehensive list of drugs and other substances which can affect the CK activity in serum is given by Young. (10.7)

### 7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

### 8.0 CALIBRATION PROCEDURES

No routine reagent calibration is necessary as the CK activity is calculated by use of the molar absorptivity of NAD, which is taken as 6.22 at 340 nm.

### 9.0 PERFORMANCE CHARACTERISTICS

#### 9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

In this study, 20 replicates of 2 control sera were run.

Mean (U/L)	SD (U/L)	CV (%)
84	± 1.9	2.3
297	± 6.8	2.3

Between-Run

In this study, 10 runs were made using a pooled serum.

Mean (U/L)	SD (U/L)	CV (%)
84	± 3.4	4.0

#### 9.2 CORRELATION

A correlation study was done comparing this CK-NAC method and a comparative CK-NAC method. The study yielded a regression curve of 0.96 (comparative method) - 3.35.

#### 9.3 LINEARITY

This procedure is linear through 1000 U/L beyond which the specimen should be diluted with an equal volume of deionized water. Reassay the specimen and multiply the results by 2.

#### 9.4 SENSITIVITY

An absorbance change of 0.001 ΔA/min corresponds to approximately 6.6 U/L CK activity.

### 10.0 REFERENCES

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