CATACHEM

CREATINE KINASE (CK) REAGENT KIT C184-0B

Contents	Product No.	Package
CK KIT B	**C184-0B**	
CK Substrate	C184-03	6 x 1.16 g
CK Diluent	C184-04	6 x 50 mL

WORKING REAGENT PREPARATION

Bring Catachem CK Diluent to room temperature. Reconstitute the contents of one vial of Catachem CK Substrate with a portion (approx. 6-7 mL) of Catachem CK Diluent.
Mix gently and pour entire contents into the Catachem CK Diluent vial.
Rinse the Catachem CK substrate vial, if necessary, with the contents of the Catachem CK Diluent vial. Ensure complete reconstitution before use.
Label the reconstituted reagent “CK Working Reagent”.

REAGENT STORAGE AND STABILITY

Store the unopened reagent kit at 2-8°C.
When stored as directed, both unopened reagents are stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
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CREATINE KINASE (CK) REAGENT KIT C184-0B MANUAL/AUTOMATED APPLICATION

Intended Use
For **IN VITRO quantitative** determination of Creatine Kinase (Creatine Phosphokinase) in serum.

Clinical Significance
Measurements of Creatine Kinase activity are primarily used for diagnosing skeletal muscle disease, myocardial infarction, cerebrovascular accidents, muscular dystrophy, hypothyroidism, pulmonary infarctions, as well as for monitoring the causes and treatments. [1]

Method History
In 1955 Oliver [1] described a method for Creatine Kinase (EC 2.7.3.2) determination using adenosine diphosphate and creatine phosphate as substrate. Oliver's method was jointly optimized by the Scandinavian Committee on Enzymes and the German Society for Clinical Chemistry [2, 3, 4]. In this procedure N-acetylcysteine (NAC) is the thiol activator and adenosine monophosphate (AMP) and p1-p5-di (adenosine) penta-phosphate (AP5A) are added to inhibit the interference caused by the adenylate kinase activity. The Catachem Creatine Kinase procedure is based upon the recommendations of the Scandinavian Committee on enzymes and the German Society for Clinical Chemistry.

Method Principle
The Creatine Kinase enzyme catalyzes the conversion of creatine phosphate and adenosine diphosphate to creatine and ATP. The resultant ATP is quantitatively determined by coupling a hexokinase (HK) reaction and a glucose-6-phosphate (GPD) reaction to the CK reaction.

GPDH catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconic acid with concomitant reduction of NADP. The amount of NADPH produced is proportional to the CK activity present in the serum sample and its concentration is measured by the increase in absorbance at 340nm. The reaction scheme below illustrates the reactions that occur in this method.

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

$$\text{D-glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{ADP} + \text{Glucose-6-phosphate}$$

$$\text{Gluc-6-phos} + \text{NADP} \xrightarrow{\text{GPD}} \text{6 phosphogluconate} + \text{NADPH}$$

Reagent Content
When reconstituted according to the directions, the concentrations of the active ingredients in the reagents will be approximately as follows:

Creatine Kinase Reagent
Each liter contains:

Creatine Phosphate	~35.0 mmol
ADP	~2.0 mmol
NADP	~2.0 mmol
NAC	~20.0 mmol
AMP	~5.0 mmol
AP5A	~10.0 umol
D-Glucose	~20.0 mmol
Hexokinase	≥3000 U
GPD	≥2000 U
Nonreactive ingredients and stabilizer	

Creatine Kinase Diluent
Each liter contains:
Buffer
Nonreactive ingredients and stabilizer

Precautions
Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagents
Prepare the required number of vials of Creatine Kinase Working Reagent by adding the corresponding Diluent volume to the CK Reagent. Mix well for 2-3 minutes.

Reagent Storage and Stability
Store the Creatine Kinase Reagents at 2-8˚C. When stored as directed, these reagents are stable until the expiration date stated on the label. Store the Creatine Kinase Working Reagent (liquid) at 2-8˚C. When prepared and stored as directed, the Creatine Kinase Working Reagent is stable for at least seven days. The Catachem Kinase Reagents have been tested to reflect shipping conditions and are stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures up to 40°C for up to one week.

Specimen Collection and Preparation
Test sera should be fresh, clear and non-hemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In separated non-hemolyzed serum the CK enzyme concentration is stable for eight hours at 25˚C and 72 hours at 2-8˚C. [5]

Quality Control
Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC. The assay can be run using an extinction co-efficient to calculate enzyme activity (as shown below) or can be calibrated using Catachem's calibrator Catacal (C1200-10)

Interfering Substances
Several substances have been reported to alter the CK activity in serum [6, 7]. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. [8]

Expected Values [9]

Adult Human Males:	24 - 195 U/L (37˚C)
Adult Human Females:	24 - 170 U/L (37˚C)

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The analytical measuring range of this assay, as performed below, is 24 U/L to 195 U/L at 37°C when using human samples. These values serve as suggested reference points only. For veterinary samples, ranges will be dependent on the species under test. It is recommended that each laboratory establish the normal ranges for the species under study and for the geographic area in which the laboratory is located.

Procedure: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer capable of measurement at 340nm

Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1.0 ml for reagent
Pipette	0.05 ml for sample
Graduated cylinder	25 ml or 50 ml for reagent

Materials Provided

Catachem Creatine Kinase Reagent and Creatine Kinase Diluent

Analytical Parameters

Wavelength	340 nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	Rate
Reaction Time	5 minutes
Reagent Volume	1.0 ml
Sample Volume	0.05 ml
Total Volume	1.05 ml
Sample-to-reagent ratio	1:21

Assay Procedure

1. Set spectrophotometer wavelength at 340nm and zero the instrument with a cuvette containing water.
2. Pipette 1.0 ml of Working Reagent into each of two cuvettes marked “Sample” and “Control”.
3. Incubate cuvettes for 2.0 minutes at 37˚C.
4. Pipette 0.05 ml of Control or Sample into their respective cuvettes. Mix all cuvettes well.
5. Replace the cuvettes in spectrophotometer and continuously monitor the change in absorbance for at least 5 minutes.
6. Read the absorbance change per minute for both the “Control” and the “Sample”.
7. Calculate the CK concentration (U/L) in the sample(s), as shown in Results and Calculations section below.

Results And Calculations

One international unit (U/L) is defined as the amount of enzyme that catalyzes the conversion of one micromole of substrate per minute under the defined conditions:

$$\text{CK (U/L)} = \frac{\Delta \text{ OD/min} \times 1.05 \text{ ml} \times 1000}{6.22 \times \text{L (cm)} \times 0.05 \text{ ml}}$$

Where:

Delta A/Min	=	change in abs/min
1.05 ml	=	total reaction volume in ml
1000	=	converts U/ml to U/L
6.22	=	extinction coefficient
L (cm)	=	1 cm reaction cuvette
0.05 ml	=	of sample used in assay

Example: Δ OD/Min = 0.025

$$\text{CK (U/L)} = \frac{0.025 \times 1.05 \text{ ml} \times 1000}{6.22 \times 1 \text{ cm} \times 0.05 \text{ ml}} = 84.4 \text{ U/L}$$

Method Performance Characteristics

Sensitivity: 0.00023-0.00028 absorbance units(U)/L
Linear Range: 0-1200 U/L
Precision: Within-run and day-to-day precision is summarized below:

Precision Study

CK	TOTAL		WITHIN-RUN	
MEAN	SD	CV	SD	CV
U/L	U/L	%	U/L	%
110	2.60	2.40	1.70	1.50
571	12.70	2.20	5.70	1.00
1014	23.00	2.30	7.80	0.70

Correlation

A comparison of this method using an automated analyzer and a reference method based upon the recommendations of the Scandinavian Committee on Enzymes resulted in the following regression statistics.

Range:	=	24 - 2107 U/L
N:	=	120
Y:	=	0.985x + 2.4
r:	=	0.999
Sy.x:	=	8.1

References

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