Creatine Kinase Assay (CK)

Cat. No. 8618
100 Tests in 96-well plate

Introduction

Creatine kinase (CK) catalyzes the reversible phosphorylation of creatine by ATP to form phosphocreatine and ADP. CK consists of two subunits: M (muscle)- and B (brain)-subunits, and has three isoenzymes: CK-MM (found in skeletal muscle), CK-MB (found in cardiac muscle), and CK-BB (found in brain and lung). Clinically, CK levels in blood are used to test for the diagnosis and/or monitoring of myocardial infraction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, and acute renal failure, as elevated CK activity levels are common to such diseases. In this colorimetric assay, CK converts phosphocreatine and ADP to creatine and ATP, where ATP is then used for subsequent enzyme-coupled reactions to form NADPH. CK activity is determined by assaying the rate of NADPH formation, which is proportional to the reduction in absorbance at 340nm over time (ΔOD_{340nm}/min).

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8618a	1	Assay buffer	25 mL	4°C
8618b	1	CK positive control	20 µL	-20°C
8618c	1	Substrate	1.6 mL	-20°C
8618d	1	NADP	1.0 mL	-20°C
8618e	1	Cofactor	0.4 mL	-20°C
8618f	1	Enzyme mix	1.2 mL	-20°C

Product Use

The CK Assay kit measures CK activity in various types of samples, including tissue and cell lysate. This product is for research purposes only and is not approved for use in animals, humans, or diagnostic procedures.

Quality Control

Diluted CK positive control is used to measure CK activity with the CK Assay kit after various reaction times (Figure 2). The detection range is 0.008 to 0.05 U/ml when using a 96-well plate.

Shipping

Shipped on dry ice.

Preparation of Positive Control

1. Diluted CK positive control: Add 1 µl of CK positive control (8618b) into 799 µl of assay buffer (8618a). Prepare diluted CK positive control to a final volume of 10 µL/well in a 96-well flat bottom plate.

Procedures (96-well plate)

A. Preparation of test samples and blank

1. Cell or tissues can be homogenized in 4 volumes of the assay buffer (8618a). Centrifuge the samples at 10,000 ×g for 10 minutes at 4ºC to remove insoluble material. The soluble fraction may be assayed directly.
2. Samples should be serial diluted to ensure that the readings are within the range of the standard curve. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.
3. Prepare blank by adding 10 µL assay buffer (8618a) into one well of the 96-well flat bottom plate.

B. Preparation of working reagent and measurements

1. Prepare appropriate volume of CK assay working reagent based on the number of samples to be measured. For each well of reaction, prepare working reagent by mixing 48 µL assay buffer (8618a), 16 µL substrate (8618c), 10 µL NADP (8616d), 4 µL cofactor (8618e), and 12 µL enzyme mix (8618f).
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing diluted CK positive control, samples, and blank. Immediately mix the reaction and start recording OD_{340nm} over 6 minute intervals, collecting data every 1 minute. Figure 1 shows data for CK positive control.

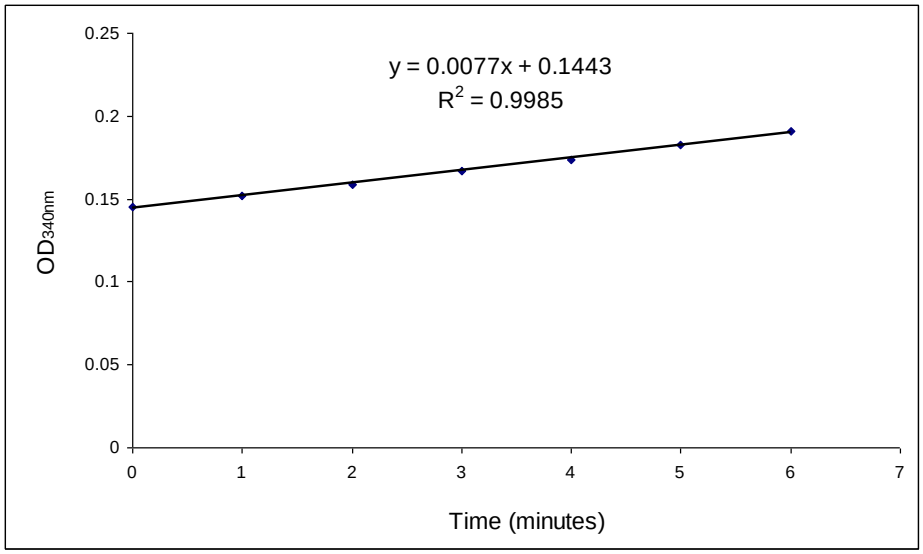

Figure 1. Change in absorbance of diluted CK positive control at 340nm.

C. Calculations

1. Determine the change in absorbance ΔOD_{340nm}/min by plotting the absorbance value at ΔOD_{340nm} as a function of reaction time, and then obtain the slope of the linear portion of the curve, as shown in Figure.2.

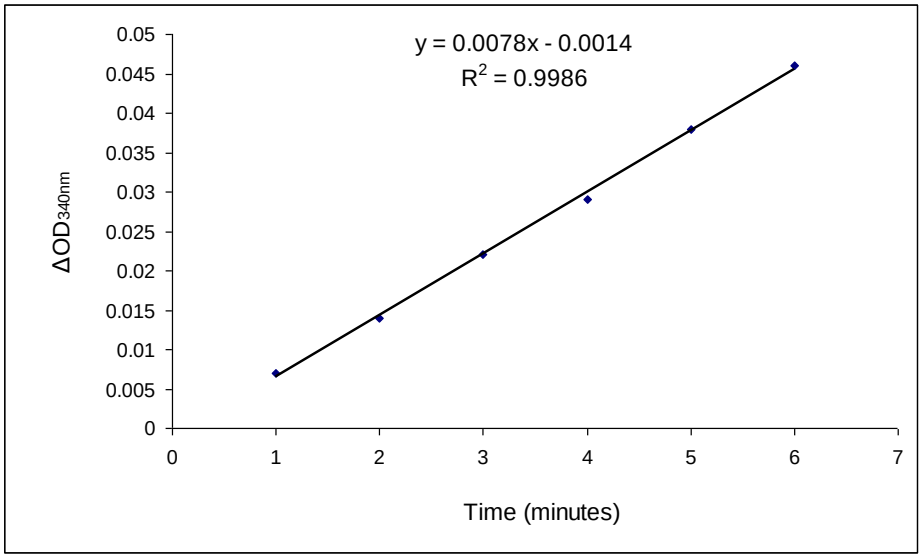

Figure 2. The change in absorbance ΔOD_{340nm} of diluted CK positive control during the time at 340nm.

2. Calculate CK activity using the following formula:

$$\text{CK (U/ml)} = \frac{\Delta OD_{340nm}/\text{min} \times 100\ \mu l}{1.94\ mM^{-1} \times 10\ \mu l} \times \text{sample dilution}$$

Note: The actual extinction coefficient for NADH at 340nm is $6.22 mM^{-1}cm^{-1}$. This value has been adjusted for the path length of the solution in a 96-well plate.

Unit definition: One unit converts 1.0 µmol of NADPH to $NADP^+$ per minute at 25 ºC, at pH 7.4.

3. Use the formula below to calculate the activity of the CK positive control:

$$\text{CK positive control (U/ml)} = \frac{0.0078 \times 100\ \mu l}{1.94\ mM^{-1} \times 10\ \mu l} \times 800 = 32.16\ \text{(U/ml)}$$