



Creatine Kinase Assay (CK)

Cat# 8618

100 Tests in 96-well plate

Product Description

The Creatine Kinase (CK) Activity Assay is a fast, straightforward, reproducible, and standardized method for quantifying total CK activity in biological samples. 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 infarction (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 increase in absorbance at 340nm over time ($\Delta OD_{340nm}/min$).

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8618a	1	Assay buffer	25 mL	4°C
8618b	1	CK positive control	20 μ L	-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

Material Supplied by User

UV Microplate reader

Clear 96-well flat-bottom plate

1X DPBS ScienCell, Cat# 0303

Product Use

The CK Assay kit measures CK activity in various types of samples, including, S plasma, 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

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.

Procedure (96-well plate)

A. 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.

B. Preparation of test samples and blank

1. Cell lysates:
 - 1.1. For attached cells, remove supernatant from cells, wash cells twice with cold 1X DPBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 $\times$ g for 10 min) making sure to remove any remaining DPBS before adding the assay buffer.
 - 1.2. Homogenize 2×10^7 cells per 1 mL of the assay buffer (8618a). Pipette up and down to resuspend cells and rock the lysates gently at 2–8 $^{\circ}\text{C}$ for 30 minutes. Centrifuge the samples at 10,000 $\times$ g for 10 minutes at 4 $^{\circ}\text{C}$ to remove insoluble material. The soluble fraction may be assayed directly.
 - 1.3. Lysates should be used immediately or aliquot and stored at -70 $^{\circ}\text{C}$. Thawed lysates should be kept on ice prior to use.
 - 1.4. Samples should be serial diluted to ensure that the readings are within the range of the standard curve
2. Plasma or serum samples:
 - 2.1. Centrifuge to remove insoluble materials. No dilution is recommended for serum/plasma samples.
3. Prepare test samples to a final volume of 10 μL /well on the 96-well flat bottom plate.
4. Prepare blank by adding 10 μL assay buffer (8618a) into one well of the 96-well flat bottom plate.

C. 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 interval, collecting data every 1 minute. Figure 1 shows data for CK positive control.

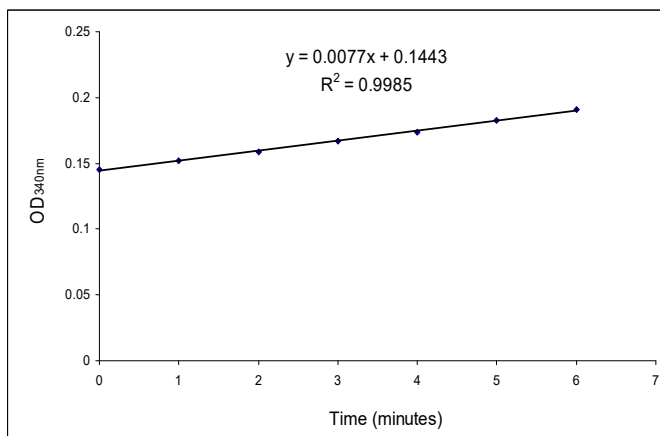


Figure 1. Change in absorbance of diluted CK positive control at 340nm.

D. Calculations

1. Calculate the mean absorbance for each set of duplicate samples and positive controls. Subtract the blank average absorbance for each minute.
2. Determine the change in absorbance $\Delta OD_{340nm}/min$ 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.

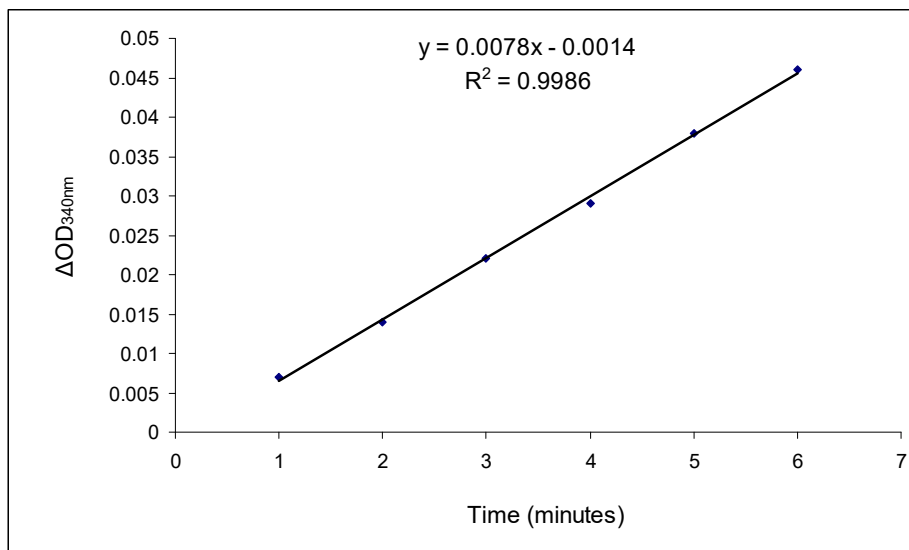


Figure 2. The change in absorbance ΔOD_{340nm} of diluted CK positive control during the time at 340nm.

3. Calculate CK activity using the following formula:

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

$\Delta OD_{340nm}/min$: Average absorbance change/minute.

1.94 mM^{-1} is the adjusted path length of the solution in a 96-well plate. The actual extinction coefficient for NADH at 340nm is $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$.

$10 \mu l$: The volume of the sample or positive control added into each well.

Unit definition: One unit converts $1.0 \mu \text{mol}$ of NADPH to NADP^+ per minute at 25°C , at pH 7.4.

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

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