Creatine Kinase (CK) Activity Assay Kit

Catalog No: E-BC-K558-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 3.7 U/L

Detection range: 3.7-160 U/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure Creatine kinase (CK) activity in serum (plasma), animal tissue, culture cells samples.

▲Background

Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phosphocreatine kinase, is an enzyme (EC 2.7.3.2) expressed by various tissues and cell types. It can transfer phosphate group from phosphocreatine to ADP and catalyze the production of ATP. Creatine kinase plays a key role in cell energy metabolism.

▲Detection principle

Creatine kinase (CK) catalyze creatine phosphate and ADP to produce creatine and ATP. Hexokinase catalyze creatine and glucose to produce glucose-6-phosphate. Glucose-6-phosphate dehydrogenase (G-6-PD) catalyze glucose-6-phosphate and NADP+ to produce NADPH which have a maximum absorption peak at 340 nm. The CK activity can be calculated by measuring the OD values at 340 nm.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Enzyme Solution	60 mL × 2 vials	2-8℃ , 3 months, , shading light
Reagent 2	Acid Solution	12 mL × 1 vial	2-8℃ , 3 months, shading light
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (340 nm), Micropipettor, Incubator, Vortex mixer

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. Samples should not contain Fe^{3+} and Cu^{2+}, or it will inhibit the activity of G-6-PD.
2. Avoid moderate or severe hemolysis of samples. When the degree of hemolysis is high, red blood cells will release AK, ATP, G-6-PD and so on, which will affect the results.
3. The activity of CK is unstable, temperature and light may lead to the loss of enzyme activity. So the sample should be detect as soon as possible after collection, or preserve the samples on ice for before detection.
4. When the reagent 1 is taken, it is recommended to avoid the contamination of reagent.

Pre-assay preparation

▲ Reagent preparation

Preheat the reagent 2 in 37℃ for 10 min before use.

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (3.7-160 U/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Human serum	1
Human plasma	1
Mouse serum	1
Rat serum	1
HepG2 cells homogenization	1
10% Rat kidney tissue homogenization	1
10% Rat brain tissue homogenization	2-5
10% Rat liver tissue homogenization	2-10

Note: The diluent is PBS (0.01 M, pH 7.4).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	340 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

The measurement of samples

(1) Blank tube: Take 1000 μL of reagent 1, 50 μL of double distilled water to the EP tube.
Sample tube: Take 1000 μL of reagent 1, 50 μL of sample to the EP tubes.

(2) Mix fully and incubate at 37℃ for 5 min.

(3) Add 100 μL of preheated reagent 2.

(4) Mix fully and incubate at 37℃ for 2 min.

(5) Set the spectrophotometer to zero with blank tube and measure the absorbance at 340 nm with 1 cm optical path quartz cuvette (the quartz cuvette need to be preheat in 37℃ for 10 min) at initial absorbance (A1) and 5 min (A2), respectively. Calculate the ΔA = A1- A2.

▲Operation table

	Blank tube	Sample tube
Reagent 1 (μL)	1000	1000
Double distilled water (μL)	50	
Sample (μL)		50
Mix fully and incubate at 37℃ for 5 min.		
Reagent 2 (μL) (preheat in 37℃ for 10 min)	100	100
Mix fully and incubate at 37℃ for 2 min. Set the spectrophotometer to zero with blank tube and measure the absorbance at 340 nm with 1 cm optical path quartz cuvette (preheat in 37℃ for 10 min) at initial absorbance (A1) and 5 min at 37℃ (A2), respectively. Calculate the ΔA=A2-A1.		

▲Calculation

1. Serum/plasma:

Definition: The amount of CK in 1 L of serum or plasma that catalyze 1 μmol of NADPH consumed per minute is defined as 1 unit.

$$\text{CK activity (U/L)} = \frac{\triangle A}{t \times 1 \times \varepsilon} \times \frac{V_{total}}{V_{sample}} \times f$$

2. Tissue:

Definition: The amount of CK in 1 g tissue protein that catalyze 1 μmol of NADPH consumed per minute is defined as 1 unit.

$$\text{CK activity (U/gprot)} = \frac{\triangle A}{t \times 1 \times \varepsilon} \times \frac{V_{total}}{V_{sample}} \div Cpr \times f$$

Note:

ΔA: A2-A1.

t: reaction time, 5 min.

1: optical path of the quartz cuvette, 1 cm.

ε: molar extinction coefficient of NADPH at 340 nm, 6.22×10^{-3} L/(μmol•cm).

V_{sample}: volume of sample added into the reaction system, 0.05 mL.

V_{total}: volume of the added extract solution, 1.15 mL.

Cpr: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 3 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	3.7-160 U/L	Average intra-assay CV (%)	5.2
Sensitivity	3.7 U/L	Average inter-assay CV (%)	8.1

▲Example analysis

Dilute 10% rat heart tissue homogenate with PBS (0.01 M, pH 7.4) for 2 times, take 50 μL of diluted sample, then carry the assay according to the operation table.

The results are as follows:

the average OD value of sample at 0 min is 0.474, the average OD value of sample at 5 min is 0.681, the concentration of protein in sample is 6.13 g/L, and the calculation result is:

$$CK(U/gprot) = (0.681-0.474) \div 5 \times 1 \times 6.22 \times 10^{-3} \times 1.15 \div 0.05 \div 6.13 \times 2$$

$$=49.95\ (U/gprot)$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection.

▲ Tissue

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).

▲ Cells

Collect the cells and wash the cells with homogenization medium for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or 10 mM Tris-buffer (pH 7.4) including 0.25 M sucrose, 1 mM EGTA, 1 mM mercaptoethanol.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.