（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K558-S

Specification：50 Assays(48 samples)/100 Assays(96 samples)

Measuring instrument：Spectrophotometer（340 nm）

Detection range：3.7-160 U/L

Elabscience® Creatine Kinase (CK) Activity Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

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

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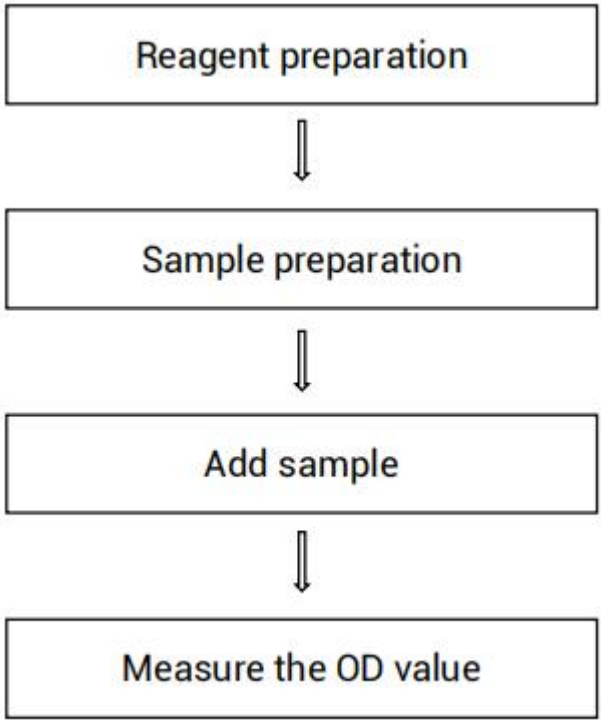
Assay summary



Intended use

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

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	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Enzyme Solution	60 mL × 1 vial	60 mL × 2 vials	2-8℃, 12 months shading light
Reagent 2	Acid Solution	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

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

Reagent preparation

Preheat the acid solution in 37℃ for 10 min before use. Equilibrate other reagents to room temperature before use.

Sample preparation

① **Sample preparation**

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation

1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor

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 enzyme solution is taken, it is recommended to avoid the contamination of reagent.

Operating steps

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

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

③ Add 100 μL of preheated acid solution.

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 (A_1) and 5 min (A_2), respectively. Calculate the $\triangle A=A_1-A_2$.

Calculation

The sample:

1. Serum (plasma) sample:

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.

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

2. Tissue sample and cells sample:

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

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

[Note]

△A: A_2-A_1.

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.

C_{pr}: Concentration of protein in sample (gprot/L).

f: Dilution factor of sample before tested

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	8.4	26.8	95.5
%CV	5.6	4.9	5.1

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	8.4	26.8	95.5
%CV	8.4	7.9	8.0

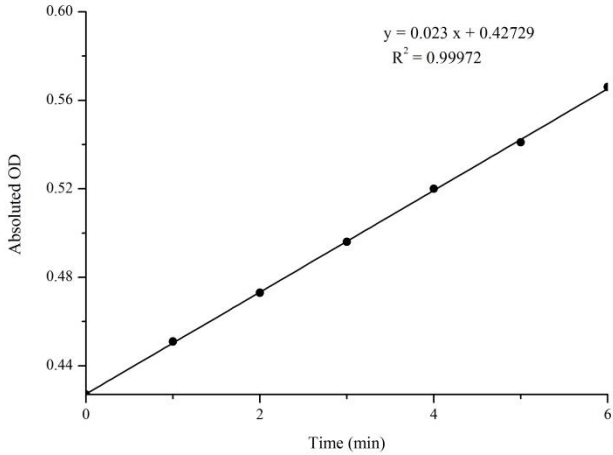
Sensitivity

The analytical sensitivity of the assay is 3.7 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

Take rat muscle as an example, take 2% rat muscle homogenate 50μL, operate according to the operation steps, record the OD value A_1, react at 37℃, measure the measuring tube every minute, record the OD value：

Reaction time (min)	0	1	2	3	4	5	6
Absoluted OD	0.427	0.451	0.473	0.496	0.520	0.541	0.566



Appendix Π Example Analysis

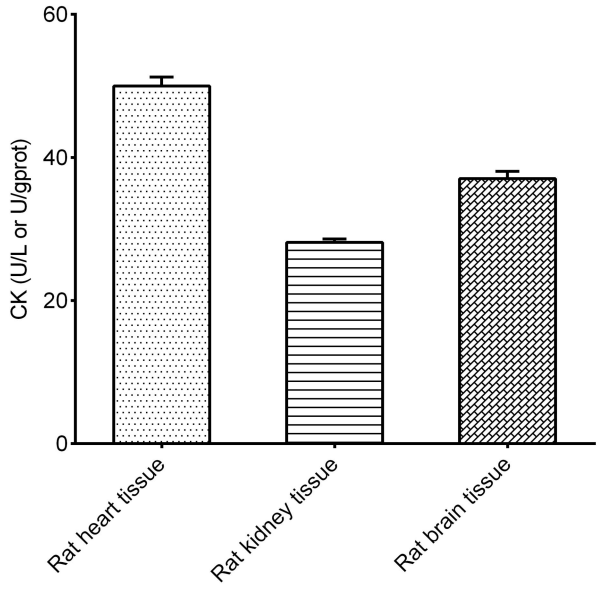
Example analysis：

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

$$\text{CK (U/gprot)} = \frac{0.681 - 0.474}{5 \times 1 \times 6.22 \times 10^{-3}} \times \frac{1.15}{0.05} \div 6.13 \times 2 = 49.95 \text{ (U/gprot)}$$

Detect 10% rat heart tissue homogenate (the concentration of protein is 6.13 g/L, dilute for 2 times), 10% rat kidney tissue homogenate (the concentration of protein is 7.84 g/L), 10% rat brain tissue homogenate (the concentration of protein is 4.66 g/L, dilute for 2 times) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.