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

Catalog No：E-BC-K558-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader（320-360 nm）

Detection range：13.55-115.86 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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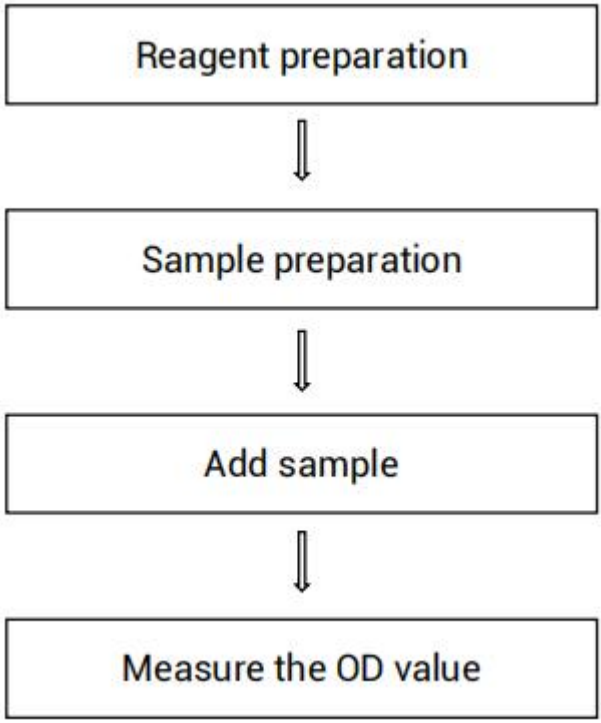
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Assay summary



Intended use

The kit can be used to detect the activity of creatine kinase (CK) in serum, plasma, animal tissue and cell samples.

Detection principle

Creatine kinase (CK, EC 2.7.3.2) catalyze creatine phosphate and ADP to produce creatine and ATP. Hexokinase catalyze ATP and glucose to produce glucose-6-phosphate. Glucose-6-phosphate dehydrogenase (G6P-DH , Glucose-6-phosphate dehydrogenase) 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(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Solution	14 mL × 1 vial	28 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Acid Solution	4 mL × 1 vial	8 mL × 1 vial	2-8℃, 12 months, shading light
	UV-Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Microplate reader (320-360 nm, optimum wavelength: 340 nm), Incubator (37℃), Vortex mixer

Reagents:

Double distilled water, PBS (0.01 mol/L, pH 7.4)

Reagent preparation

Acid solution should be incubated at 37°C for 10 min before use.

Equilibrate other reagents to room temperature before use.

Sample preparation

① **Sample preparation:**

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

Tissue samples:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 mol/L, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ 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).

Cell (adherent or suspension) samples:

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 mol/L, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes to at 4℃ 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
10% Rat kidney tissue homogenate	1
10% Rat brain tissue homogenate	2-5
10% Rat liver tissue homogenate	2-10
1×10^6 HepG2 cells	1

Note: The diluent is PBS (0.01 mol/L, 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 Serum and plasma samples must be clarified.
② Preserve the sample on ice during use.
③ It is recommended to aliquot enzyme solution into smaller quantities to avoid contamination.

Operating steps

① Blank well: add 10 μL of double distilled water into blank wells.
Sample well: add 10 μL of sample into sample wells.
② Add 200 μL of enzyme solution to each well.
③ Mix fully for 5 s with microplate reader and incubate at 37℃ for 5 min.
④ Add 20 μL of acid solution which is incubated at 37°C for 10 min to each well.
⑤ Mix fully and incubate at 37℃ for 2 min, measure the OD value of each well at 340 nm, recorded as A_1.
⑥ Incubate at 37℃ for 5 min. Measure the OD values of each well at 340 nm with microplate reader, recorded as A_2. $\triangle A = A_2 - A_1$.

Calculation

1. **Serum (plasma) sample**

   Definition: The amount of creatine kinase (CK) in 1 L serum or plasma sample that hydrolyze the substrate to produce 1 μmol NADPH in 1 minute at 37°C is defined as 1 unit.

$$\text{CK activity (U/L)} = \frac{\triangle A}{t \times 0.6 \times \varepsilon} \times \frac{V_1}{V_2} \times f$$

2. **Tissue and cell samples**:

   Definition: The amount of creatine kinase (CK) in 1 g tissue or cell sample that hydrolyze the substrate to produce 1 μmol NADPH in 1 minute at 37°C is defined as 1 unit.

$$\text{CK activity (U/gprot)} = \frac{\triangle A}{t \times 0.6 \times \varepsilon} \times \frac{V_1}{V_2} \div C_{pr} \times f$$

[Note]

ΔA : $\Delta A = A_2 - A_1$.

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

0.6 : Optical path, 0.6 cm.

V_1 : The volume of the reaction system, 0.23 mL.

V_2 : The volume of the sample, 0.01 mL.

T: The reaction incubation time, 5 min.

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

f: Dilution factor of the 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(U/L)	25.00	34.00	78.00
%CV	2.5	5.4	7.4

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean(U/L)	25.00	34.00	78.00
%CV	3.4	6.3	9.6

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc.(U/L)	25	34	78
Observed Conc. (U/L)	25	34.6	82
Recovery rate (%)	100	102	105

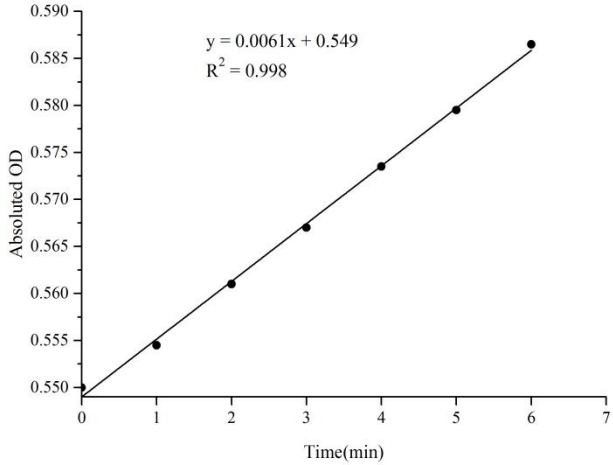
Sensitivity

The analytical sensitivity of the assay is 6.16 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. Sample standard curve

For example, take 10 μL of 10% rat liver tissue homogenate and carry the assay according to the operation steps. After recording the OD value A_1, measure the dynamics for 6 min, measure the measuring tube every minute, and record the OD value. The results are as follows:

Time(min)	0	1	2	3	4	5	6
OD value	0.550	0.554	0.561	0.567	0.573	0.579	0.586



Appendix Π Example Analysis

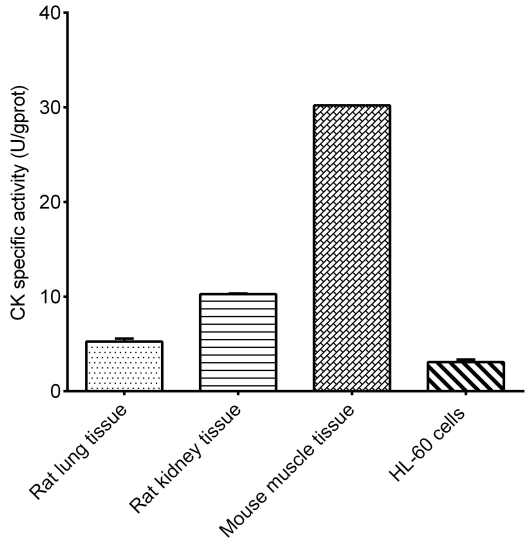
Example analysis：

Take 10 μL of 10% rat lung tissue homogenate and carry the assay according to the operation steps. The results are as follows:

The A_1 of sample well is 0.404, the A_2 of sample well is 0.438, the concentration of protein in sample is 7.97 g/L, and the calculation result is:

$$\text{CK activity (U/gprot)} = \frac{0.438 - 0.404}{5 \times 0.6 \times 6.22 \times 10^{-3}} \times \frac{0.23}{0.01} \div 7.97 = 5.26\ (\text{U/gprot})$$

Detect 10% rat lung tissue homogenate (the concentration of protein is 7.97 gprot/L), 10% rat kidney tissue homogenate (the concentration of protein is 8.82 gprot/L), 10% mouse muscle tissue homogenate (the concentration of protein is 3.51 gprot/L) and 1×10^6 HL-60 cells (the concentration of protein is 10.85 gprot/L) 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.