Creatine Kinase (CK) Activity Assay Kit

Catalogue No.: K118

Size: 48T(46S)/ 96T(94S)

Range: 13.55-115.86 U/L

Sensitivity: 6.16 U/L

Kit component:

Item	Component	Size（48T）	Size（96T）	Storage
Reagent 1	Enzyme Solution	14 mL	28 mL	2-8℃, shading light
Reagent 2	Acid Solution	4 mL	8 mL	2-8℃, shading light
	Product Description	1 copy		

Storage:

The kit should be stored at 2-8℃ shading light for 3 months.

Principle of the Assay:

Creatine kinase (CK, EC 2.7.3.2) catalyze creatine phosphate and ADP to produce creatine and ATP. Hexokinase catalyze creatine 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.

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

Assay Procedure (For reference):

1. **Reagent preparation**

Bring all reagents to room temperature before use. Acid solution should be incubated at 37°C for 10 min before use.

2. **Sample preparation**

a. **Preparation of sample**

Serum and plasma: detect directly.

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 M, 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 (K001).

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 M, 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 (K001).

b. **Dilution of sample**

Before the formal test, 2-3 samples with significant differences should be selected and diluted into different concentrations for pre-test. 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).

3. Add samples and test

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 A1.

⑥ Incubate at 37℃ for 5 min. Measure the OD values of each well at 340 nm with microplate reader, recorded as A2. △A = A2 - A1.

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.

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

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.

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

[Note]

ΔA : ΔA = A2 – A1.

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

0.6 : Optical path, 0.6 cm.

V1 : The volume of the reaction system, 0.23 mL.

V2 : The volume of the sample, 0.01 mL.

t: The reaction incubation time, 5 min.

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

f: Dilution factor of the sample before tested.

Notes:

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 a lab coat and latex gloves during the experiment.

4. The detection range of the kit is not equivalent to the concentration range of the substance to be measured in the sample. 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. FineTest 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.