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

Catalog No：E-BC-F154

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：1.17-100.00 μmol/L

Elabscience® Phosphocreatine (PCr) Fluorometric 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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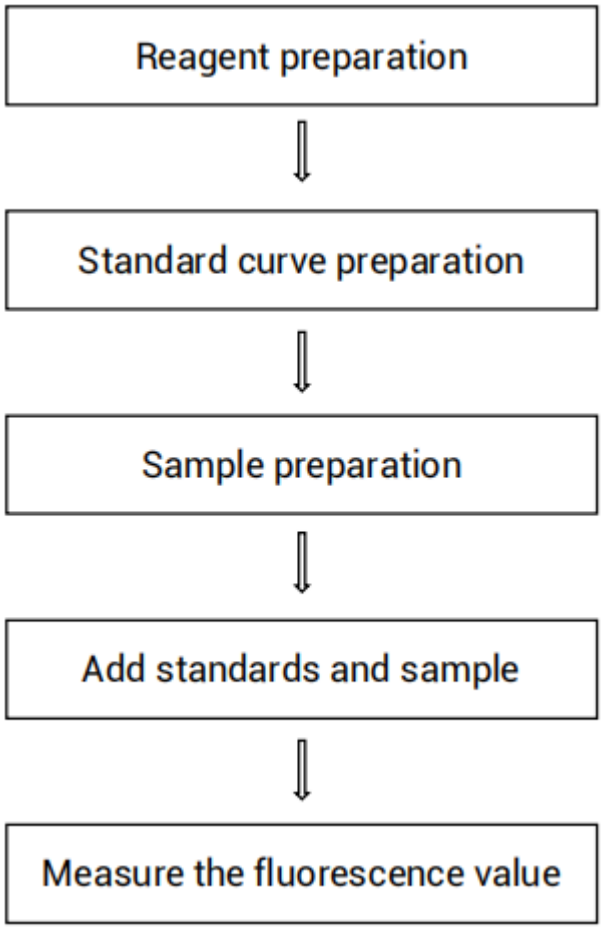
Assay summary



Intended use

This kit can be used to measure phosphocreatine (PCr) content in animal tissue and cell samples.

Detection principle

Phosphocreatine (PCr) is a high-energy phosphate compound that mainly exists in muscle and nerve tissues and is an important substance in the rapid energy supply system of living organisms. It plays a buffering and energy storage role in cellular energy metabolism through its interaction with adenosine triphosphate (ATP), especially providing immediate energy to muscles during short-term high-intensity exercise.

The detection principle of this kit: The products generated by PCr through a series of enzymatic reactions cause the fluorescent probe to produce fluorescence. Fluorescence can be detected at an excitation wavelength of 535 nm and an emission wavelength of 587 nm. The concentration of PCr in the sample is calculated based on the magnitude of the fluorescence value.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution A	45 mL × 1 vial	-20°C, 12 months shading light
Reagent 2	Extraction Solution B	0.45 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Buffer Solution	15 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Substrate	0.1 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	Co-factor	Powder × 2 vials	-20°C, 12 months shading light
Reagent 6	Enzyme Reagent	9.7 mL×1 vial	-20°C, 12 months shading light
Reagent 7	Accelerant	1.2 mL×1 vial	-20°C, 12 months shading light
Reagent 8	Fluorescence Probe	5 mL×1 vial	-20°C, 12 months shading light
Reagent 9	10 mmol/L Standard Solution	0.2 mL×1 vial	-20°C, 12 months shading light
	Black Microplate	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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator

Consumptive material:

10kDa MWCO Spin Filter (Inner tube 0.5 mL, outer tube 1.5 mL)

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of extraction working solution:
Before testing, please prepare sufficient extraction working solution. For example, prepare 500 μL of extraction working solution (mix well 495 μL of extraction solution A and 5 μL of extraction solution B). Keep it on ice during use protected from light and used up within 8 h.

③ The preparation of substrate working solution:
Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 300 μL of substrate working solution (mix well 5 μL of substrate and 295 μL of buffer solution). Keep it on ice during use protected from light. Store at -20°C for 2 days protected from light.

④ The preparation of co-factor working solution:
Dissolve one vial of co-factor with 0.75 mL of buffer solution, mix well to dissolve. Keep it on ice during use protected from light. Store at -20°C for 3 days protected from light.

⑤ The preparation of reaction working solution:
For each well, prepare 100 μL of reaction working solution (mix well 12 μL of co-factor working solution and 88 μL of enzyme reagent). Keep it on ice during use protected from light. Store at -20°C for 2 days protected from light.

⑥ The preparation of 100 μmol/L standard solution:
Before testing, please prepare sufficient reaction working solution. For example, prepare 1000 μL of 100 μmol/L standard solution (mix well 10 μL of 10 mmol/L standard solution and 990 μL of buffer solution). The standard solution should be prepared on spot protected from light and used up within 1 day.

⑦ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 70, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**20**	**40**	**50**	**70**	**80**	**100**
100 μmol/L Standard (μL)	0	20	40	80	100	140	160	200
Buffer Solution (μL)	200	180	160	120	100	60	40	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).

② Homogenize 50 mg tissue in 450 μL extraction working solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material.

④ Collect 200-400 μL supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4°C.

⑤ Take the filtered sample supernatant and preserve it on ice for detection. The supernatant should be used up within 4 h (Samples need to be stored at 4°C, as temperature can affect the test results).

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 3 × 10^6 cells).

② Homogenize 3 × 10^6 cells in 600 μL extraction working solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material.

④ Collect 200-400 μL supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4°C.

⑤ Take the filtered sample supernatant and preserve it on ice for detection. The supernatant should be used up within 4 h (Samples need to be stored at 4°C, as temperature can affect the test results).

② **Dilution of sample**

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

Sample type	Dilution factor
10% Mouse kidney tissue homogenate	1
10% Mouse muscle tissue homogenate	1-4
10% Mouse brain tissue homogenate	1-3
1×10^6 RAW 264.7 cells	1

Note: The diluent is extraction working solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 20 μL of standard solution with different concentrations into the wells.
Sample well: add 20 μL of sample into the wells.
Control well: add 20 μL of sample into the wells.

② Add 20 μL of substrate working solution into each well.

③ Add 100 μL of reaction working solution into each well.

④ Add 20 μL of accelerant into standard wells and sample wells. Add 20 μL of double distilled water into control wells.

⑤ Add 40 μL of fluorescence probe into each well.

⑥ Mix fully with fluorescence microplate for 5s. Incubate at 37°C for 20 min protected from light. Measure the fluorescence at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, as F.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Tissue samples:

$$\text{PCr content (μmol/kg wet weight)} = (\Delta F - b) \div a \div m \times V \times f$$

2. Cell sample:

$$\text{PCr content (μmol/10^6)} = (\Delta F - b) \div a \div n \times V \times f$$

[Note]

ΔF: $\Delta F = F_{sample} - F_{control}$.

m: The weight of tissue, kg.

V: The volume of extraction working solution, L.

n: The number of cell sample/10^6.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse heart tissue 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)	20.00	50.00	80.00
%CV	1.3	1.6	3.5

Inter-assay Precision

Three mouse heart tissue were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	20.00	50.00	80.00
%CV	8.3	9.2	9.7

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 96%.

	Sample 1	Sample 2	Sample 3
Expected Conc(μmol/L)	20.00	50.00	80.00
Observed Conc(μmol/L)	18.8	48.0	78.4
Recovery rate (%)	94.0	96.0	98.0

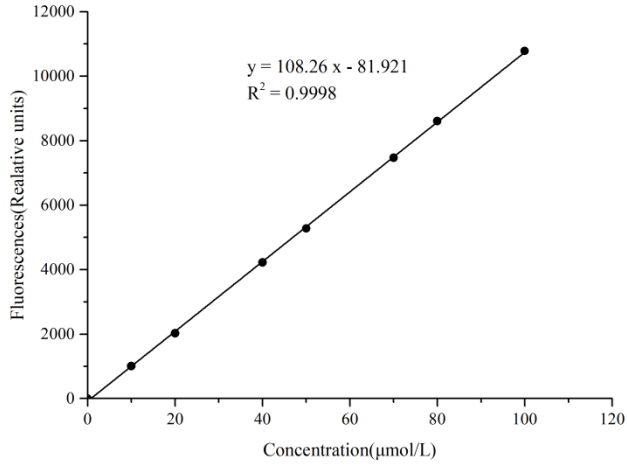
Sensitivity

The analytical sensitivity of the assay is 1.17 μmol/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:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	0	10	20	40	50	70	80	100
F value	2646	3621	4729	6885	8019	10079	11184	13391
	2658	3701	4623	6863	7848	10164	11341	13479
Average F value	2652	3661	4676	6874	7934	10122	11263	13435
Absoluted F value	0	1009	2024	4222	5282	7470	8611	10783



Appendix Π Example Analysis

Example analysis：

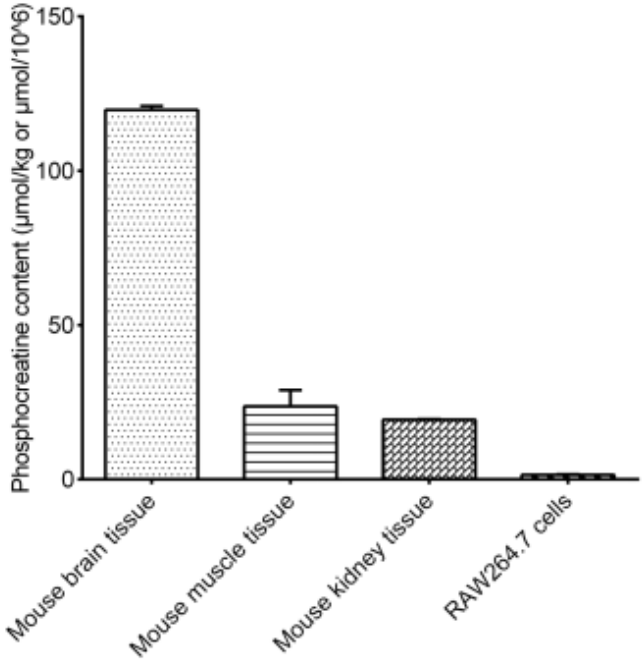
Take 20 μL of 10% mouse brain tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 108.26 x - 81.921, the F value of the sample well is 5245, the F value of the control well is 3887, ΔF = 5245 - 3887 = 1358. The calculation result is:

$$\text{PCr content (μmol/kg wet weight)} = (1358 + 81.921) \div 108.26 \div 0.0001 \times 0.0009$$

$$= 119.71 \text{ μmol/kg wet weight}$$

Detect 10% mouse brain tissue homogenate, 10% mouse muscle tissue homogenate, 10% mouse kidney tissue homogenate and 1×10^6 RAW264.7 cells, 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.