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

Catalog No：E-BC-K900-M

Specification：48T (32 samples)/96T (80 samples)

Measuring instrument：Microplate reader(510-520 nm)

Detection range：0.04-1 mmol/L

Elabscience® Creatine Colorimetric Assay Kit (Enzyme Method)

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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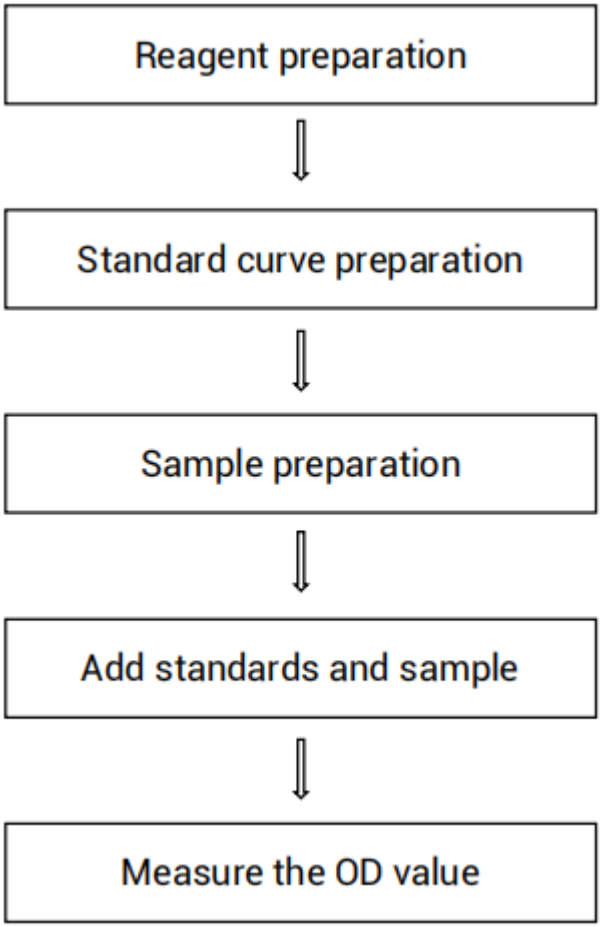
Assay summary



Intended use

This kit can be used to measure creatine content in serum, plasma, urine and animal tissue samples.

Detection principle

Creatine is a nitrogen-containing organic acid that can assist in providing energy for muscle and nerve cells. Creatine is mainly stored in muscle tissue. It can relieve muscle fatigue and tension, enhance muscle elasticity, make muscles firmer, accelerate the synthesis of human proteins, lower cholesterol, blood lipids and blood sugar, and delay the aging of the body. Creatine is catalyzed by enzymes to produce hydrogen peroxide. Hydrogen peroxide reacts with a chromogenic agent to form a pink substance. The content of creatine can be calculated by measuring the change in OD value at a wavelength of 515 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	55 mL × 1 vial	55 mL × 2 vials	2-8°C, 12 months
Reagent 2	Chromogenic Agent A	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Chromogenic Agent B	3.5 mL × 1 vial	7 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	1 mmol/L Standard	1.5 mL × 1 vial	1.5 mL × 2 vials	2-8°C, 12 months, shading light
	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 (510-520 nm, optimum wavelength: 515 nm), Incubator, Homogenizer, Centrifuge

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of standard curve：

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

Dilute 1 mmol/L standard solution with extraction solution to a serial concentration, the recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**1**
1 mmol/L standard (μL)	0	20	40	60	80	120	160	200
Extraction solution (μL)	200	180	160	140	120	80	40	0

Sample preparation

① Sample preparation

Serum, plasma and urine samples:

Take 20 μL of sample and 20 μL of extraction solution, mix well. Keep it on ice for detection and used up within 8 h.

Tissue sample:

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

② Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used up within 8 h.

② Dilution of sample

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

Sample type	Dilution factor
Mouse serum	1-2
Rat serum	1-2
Human serum	1
Urine	5-10
10% Mouse muscle tissue homogenate	3-5
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1

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

Operating steps

① Standard well: Add 10 μL of standard solution with different concentrations into the wells.
Sample well: Add 10 μL of samples into wells.

② Add 180 μL of chromogenic agent A into each well.

③ Mix fully and incubate at 37°C for 5 min.

④ Measure the OD value of each well at 515 nm, as A_1.

⑤ Add 60 μL of chromogenic agent B into each well.

⑥ Mix fully and incubate at 37°C for 5 min.

⑦ Measure the OD value of each well at 515 nm, as A_2. $\Delta A = A_2 - A_1$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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. Serum (plasma) or urine samples:

$$\text{Creatine content (μmol/L)} = (\Delta A_{515} - b) \div a \times 2^{*} \times 1000^{*} \times f$$

2. Tissue samples:

$$\text{Creatine content (μmol/g wet weight)} = (\Delta A_{515} - b) \div a \div m \times V \times f$$

[Note]

ΔA_{515}: $\Delta A_{515} = \Delta A_{sample} - \Delta A_{blank}$.

2*: Dilution factor of liquid sample in the preparation step.

m: The weight of sample, g.

V: The volume of extraction solution in the preparation step of tissue, mL.

1000*: 1 mmol/L=1000 μmol/L

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse 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 (mmol/L)	0.25	0.55	0.85
%CV	0.4	0.3	0.7

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.25	0.55	0.85
%CV	7.3	6.6	3.6

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 99.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.25	0.55	0.85
Observed Conc. (mmol/L)	0.24	0.55	0.88
Recovery rate (%)	96	100	103

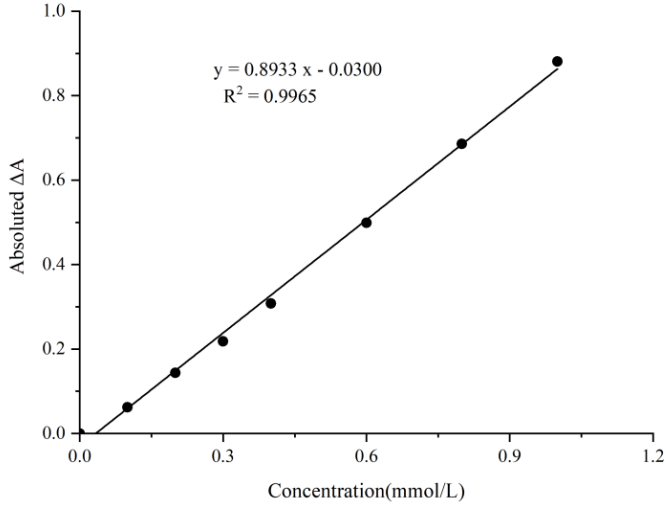
Sensitivity

The analytical sensitivity of the assay is 0.01 mmol/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 OD 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 (mmol/L)	0	0.1	0.2	0.3	0.4	0.6	0.8	1
A_1 value	0.046	0.047	0.046	0.048	0.046	0.046	0.045	0.046
	0.047	0.046	0.054	0.046	0.046	0.046	0.047	0.046
Average A_1 value	0.046	0.046	0.050	0.047	0.046	0.046	0.046	0.046
A_2 value	0.055	0.114	0.199	0.277	0.364	0.552	0.744	0.941
	0.056	0.121	0.206	0.271	0.362	0.556	0.738	0.931
Average A_2 value	0.056	0.118	0.202	0.274	0.363	0.554	0.741	0.936
Average Δ A value	0.009	0.071	0.152	0.227	0.317	0.508	0.695	0.890
Absolute Δ A value	0	0.062	0.144	0.218	0.308	0.499	0.686	0.881



Appendix II Example Analysis

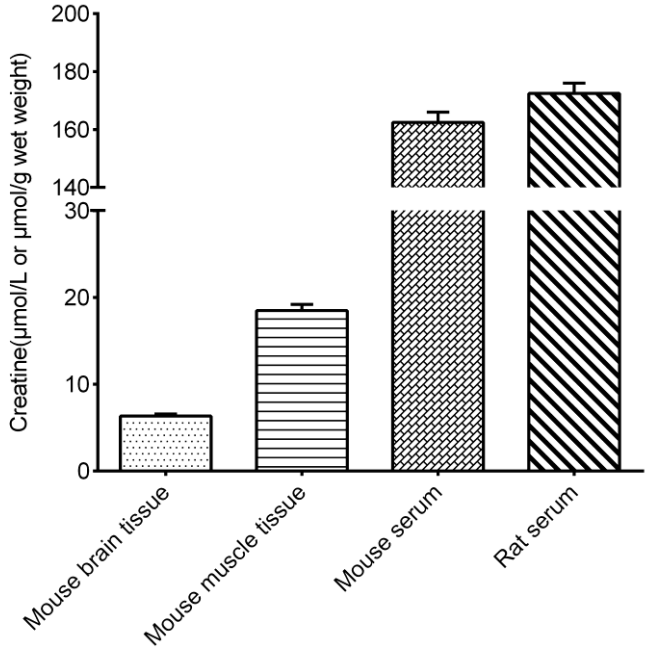
Example analysis：

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

Standard curve: y = 0.8933 x - 0.0300, the ΔA value of the blank well is 0.009, the ΔA value of the sample well is 0.608. The calculation result is:

Creatine content (μmol/g wet weight) = (0.608 - 0.009 + 0.0300) ÷ 0.8933 ÷ (0.1 ÷ 0.0009) × 1000 = 6.34 μmol/g wet weight

Detect 10% mouse brain tissue homogenate, 10% mouse muscle tissue homogenate (dilute 5 times), mouse serum (dilute 2 times) and rat serum (dilute 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.