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

Catalog No：E-BC-K157-M

Specification：48T(22 samples)/96T(46 samples)

Measuring instrument：Microplate reader（630-640 nm）

Detection range：0.03-1.5 mmol/L

Elabscience® ATP Colorimetric 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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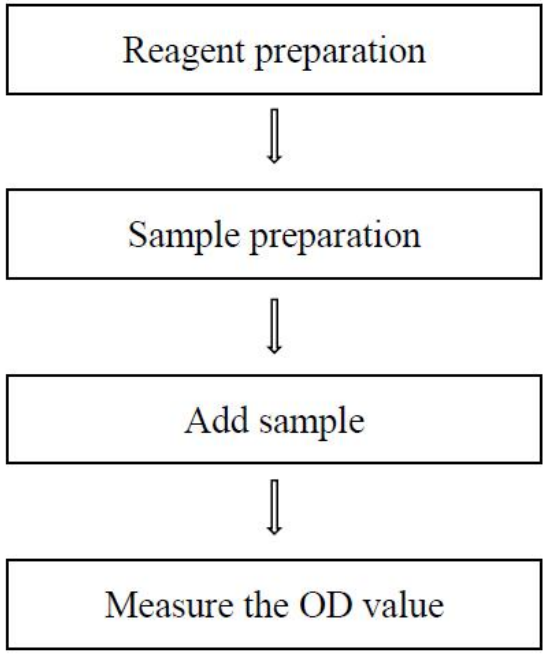
Assay summary



Intended use

This kit can be used to measure ATP content in animal tissue samples.

Detection principle

Creatine kinase catalyzes adenosine triphosphate and creatine to produce creatine phosphate. The content of phosphocreatine was determined by colorimetric method to reflect the content of ATP.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months
Reagent 3	Buffer Solution	12 mL × 1 vial	24 mL × 1 vial	2-8°C, 12 months
Reagent 4	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months
Reagent 5	Protein Precipitator	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months
Reagent 6	Chromogenic Agent A	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 7	Chromogenic Agent B	2 mL × 1 vial	4 mL × 1 vial	2-8°C, 12 months
Reagent 8	Stop Solution	6 mL× 1 vial	12 mL× 1 vial	2-8°C, 12 months
Reagent 9	Standard	Powder × 2 vials	Powder × 4 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

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 (630-640 nm, optimum wavelength: 636 nm), Micropipettor, Tubes, Vortex mixer, Incubator, 100°C Water bath, Centrifuge.

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate working solution：
Dissolve one vial of substrate with 6 mL of double distilled water in boiling water bath. If the prepared solution appear crystal before assay, please incubate in boiling water bath to dissolve fully and then store at 37°C for assay. The substrate working solution can be stored at 2-8°C for 7 days.

③ The preparation of enzyme working solution：
Dissolve one vial of enzyme reagent with 1.8 mL of double distilled water, mix well. Keep it on ice during use. Store at -20°C for 7 days.

④ The preparation of control working solution：
For each well, prepare 330 μL of control working solution (mix well 100 μL of substrate working solution, 200 μL of buffer solution and 30 μL of double distilled water). The control working solution should be prepared on spot.

⑤ The preparation of detection working solution：
For each well, prepare 330 μL of detection working solution (mix well 100 μL of substrate working solution, 200 μL of buffer solution and 30 μL of enzyme working solution). The detection working solution should be prepared on spot.

⑥ The preparation of chromogenic working solution：
For each well, prepare 100 μL of chromogenic working solution (mix well 75 μL of chromogenic agent A and 25 μL of chromogenic agent B). The

chromogenic working solution should be prepared on spot. Before using, keep the solution at 37°C for 1 hour.

⑦ The preparation of 10 mmol/L ATP standard stock solution：
Dissolve one vial of standard with 1 mL of double distilled water, mix well. Store at -20°C for 7 days.

⑧ The preparation of 1 mmol/L ATP standard solution：
For each well, prepare 30 μL of 1 mmol/L ATP standard solution (mix well 3 μL of 10 mmol/L ATP standard stock solution and 27 μL of double distilled water). Store at -20°C for 7 days.

Sample preparation

① Sample preparation

Tissue sample:

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

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 50 mg tissue in 450 μL extracting solution with a dounce homogenizer at 4°C.

④ Then incubate in boiling water bath for 2 min, cool the tubes to room temperature with running water.

⑤ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
10% Rat muscle tissue homogenate	2-4
10% Rat liver tissue homogenate	2-4
10% Rat brain tissue homogenate	2-4
10% Rat kidney tissue homogenate	2-4
10% Rat lung tissue homogenate	2-4

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

The key points of the assay

① The fresh samples should be used.

② Avoid phosphorus pollution is the key for assay, it is recommended to use disposable test tubes.

③ When the OD value is more than 1, it is necessary to increase the dilution ratio and detect again.

④ When using the chromogenic agent, should remove a portion for use to prevent the reagent pollution.

Operating steps

1. Enzymatic reaction

① Blank tube: Take 30 μL of 1 mmol/L ATP standard solution to the 1.5 mL EP tube, then add 330 μL of control working solution.

Standard tube: Take 30 μL of 1 mmol/L ATP standard solution to the 1.5 mL EP tube, then add 330 μL of detection working solution.

Control tube: Take 30 μL of sample supernatant to the 1.5 mL EP tube, then add 330 μL of control working solution.

Sample tube: Take 30 μL of sample supernatant to the 1.5 mL EP tube, then add 330 μL of detection working solution.

② Mix fully and incubate at 37°C for 30 min.

③ Add 50 μL of protein precipitator to each tube.

④ Mix fully for 3 s and centrifuge at 10000×g for 5 min, then take supernatant of each tube for detection.

2. Color reaction

① Take 60 μL of supernatant to corresponding wells.

② Add 100 μL of chromogenic working solution to each well.

③ Mix fully for 5 s with microplate reader and stand for 2 min at room temperature.

④ Add 100 μL of stop solution to each well.

⑤ Mix fully for 5 s with microplate reader, stand at room temperature for 5 min, and measure the OD value of each well at 636 nm.

Calculation

The sample:

Tissue sample:

$$\text{ATP content (mmol/kg wet weight)} = \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} \times c \div \frac{m}{V_1} \times f$$

[Note]

c: Concentration of standard (1 mmol/L)

m: The wet weight of tissue sample (g).

V_1: The volume of extracting solution in the sample pretreatment step of tissue sample.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat liver tissue 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)	0.55	0.98	1.25
%CV	5.3	5.1	4.9

Inter-assay Precision

Three rat liver tissue 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)	0.55	0.98	1.25
%CV	5.2	5.8	5.2

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 94%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.85	1.05	1.3
Observed Conc. (mmol/L)	0.8	1.0	1.2
Recovery rate (%)	99	92	91

Sensitivity

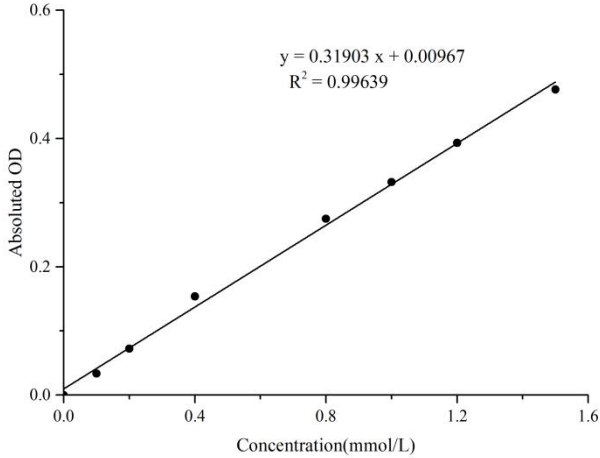
The analytical sensitivity of the assay is 0.01 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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	0.4	0.8	1.0	1.2	1.5
Average OD	0.092	0.125	0.164	0.245	0.366	0.424	0.484	0.568
Absoluted OD	0	0.033	0.072	0.153	0.274	0.332	0.392	0.476



Appendix II Example Analysis

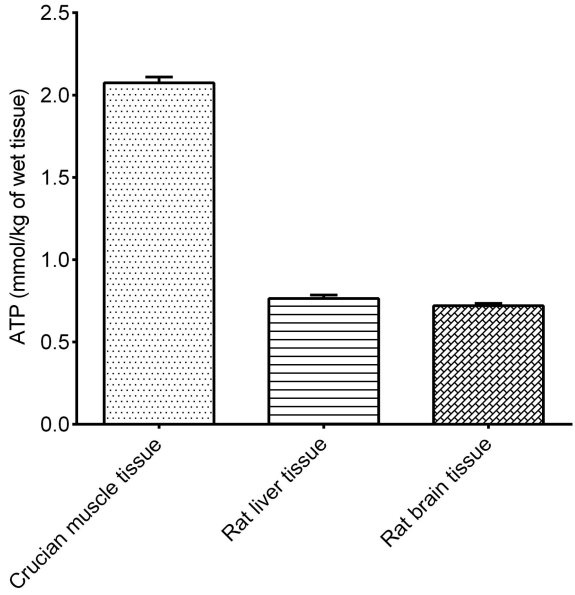
Example analysis:

For crucian muscle tissue, dilute with double distilled water for 3 times and carry the assay according to the operation steps. The results are as follows:

the average OD value of the blank is 0.104, the average OD value of the standard is 0.526, the average OD value of the sample is 0.931, the average OD value of the control is 0.899, and the calculation result is:

$$\text{ATP content (mmol/kg wet weight)} = (0.931 - 0.899) \div (0.526 - 0.104) \times 1 \div 0.1 \times 0.9 \times 3 = 2.05 \text{ mmol/kg wet weight}$$

Detect crucian muscle tissue (dilute for 3 times), rat liver tissue homogenate (dilute for 3 times), and rat brain tissue homogenate (dilute for 3 times) according to the protocol, the result is as follows:



Appendix III Publications

1. Ouyang B, Zhong Q, Ouyang P, et al. Graphene quantum dots enhance the biological nitrogen fixation by up-regulation of cellular metabolism and electron transport[J]. Chemical Engineering Journal, 2024, 487: 150694.
2. Wang Y, Wang Q, Wang X, et al. Docetaxel-loaded pH/ROS dual-responsive nanoparticles with self-supplied ROS for inhibiting metastasis and enhancing immunotherapy of breast cancer[J]. Journal of Nanobiotechnology, 2023, 21(1): 286.
3. Şahin S, Azarkan S Y, Türksoy V A. Evaluation of the effect of cannabidiol on the THLE-2 liver cell line exposed to lead[J]. Science of The Total Environment, 2024, 923: 170901.
4. Chen R, Gao S, Guan H, et al. Naringin protects human nucleus pulposus cells against TNF-α-induced inflammation, oxidative stress, and loss of cellular homeostasis by enhancing autophagic flux via AMPK/SIRT1 activation[J]. Oxidative Medicine and Cellular Longevity, 2022, 2022(1): 7655142.
5. Jin B, Gao Y, Fu Y, et al. Electroacupuncture improves cognitive function in a rat model of mild traumatic brain injury by regulating the SIRT-1/PGC-1α/mitochondrial pathway[J]. Chinese Medical Journal, 2024, 137(6): 711-719.
6. Zhang J, Zhao X, Tang J, et al. Sleep restriction exacerbates cardiac dysfunction in diabetic mice by causing cardiomyocyte death and fibrosis through mitochondrial damage[J]. Cell Death Discovery, 2024, 10(1): 446.

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.