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

Catalog No：E-BC-K157-S

Specification：50 Assays(24 samples)/100 Assays(48 samples)

Measuring instrument：Spectrophotometer（636 nm）

Detection range：0.01-1.5 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.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 4

Materials prepared by users 5

Reagent preparation 5

Sample preparation 6

The key points of the assay 7

Operating steps 8

Calculation 9

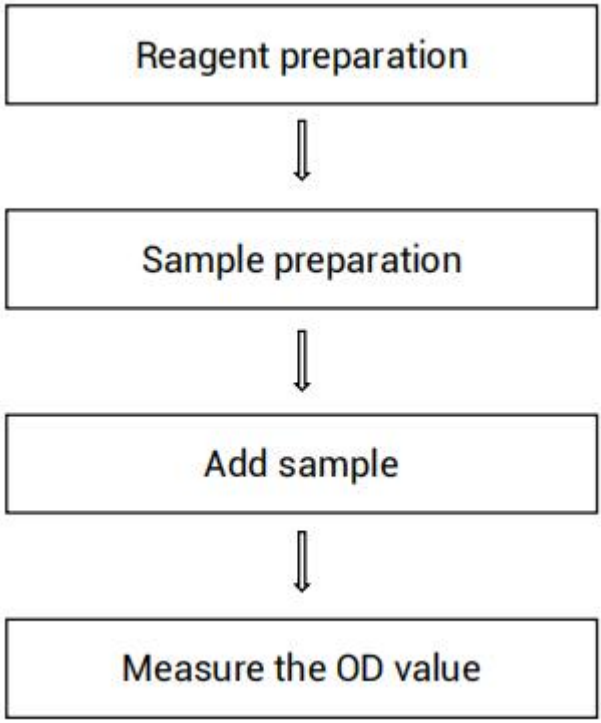
Appendix I Performance Characteristics 10

Appendix П Example Analysis 12

Appendix III Publications 13

Statement 14

Assay summary



Intended use

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

Detection principle

Creatine Kinase catalyzes adenosine triphosphate and creatine to produce creatine phosphate, then detected by phosphomolybdic acid colorimetry.

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	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	24 mL × 1 vial	48 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 7	Chromogenic Agent B	8 mL × 1 vial	16 mL × 1 vial	2-8°C, 12 months
Reagent 8	Stop Solution	30 mL× 1 vial	60 mL× 1 vial	2-8°C, 12 months
Reagent 9	Standard	Powder × 2 vials	Powder × 4 vials	2-8°C, 12 months

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:

Spectrophotometer (636 nm), Micropipettor, Incubator, Water bath, Vortex mixer, 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 tube, 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 tube, 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 tube, prepare 500 μL of chromogenic working solution (mix well 375 μL of chromogenic agent A and 125 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 tube, 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 450μL 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	2-3
10% Rat liver tissue homogenate	2-3
10% Rat brain tissue homogenate	2-3
10% Rat kidney tissue homogenate	2-3
10% Rat lung tissue homogenate	2-3

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

Operating steps

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 well 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 300 μL of supernatant to measure according to the following steps.
⑤ Add 500 μL of chromogenic working solution to each tube.
⑥ Mix fully for 5 s and stand for 2 min at room temperature.
⑦ Add 500 μL of stop solution to each tube.
⑧ Mix fully for 5 s, stand at room temperature for 5 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 636 nm wavelength with 0.5 cm optical path cuvette.

Note: cuvettes should be washed with tap water for 10 times, then washed with double distilled water for 4~5 times, avoid contaminated with phosphorus.

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)	0.47	0.95	1.20
%CV	3.7	3.6	3.5

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)	0.47	0.95	1.20
%CV	9.2	8.7	10.0

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 103%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.65	1.05	1.35
Observed Conc. (mmol/L)	0.7	1.0	1.4
Recovery rate (%)	106	98	105

Sensitivity

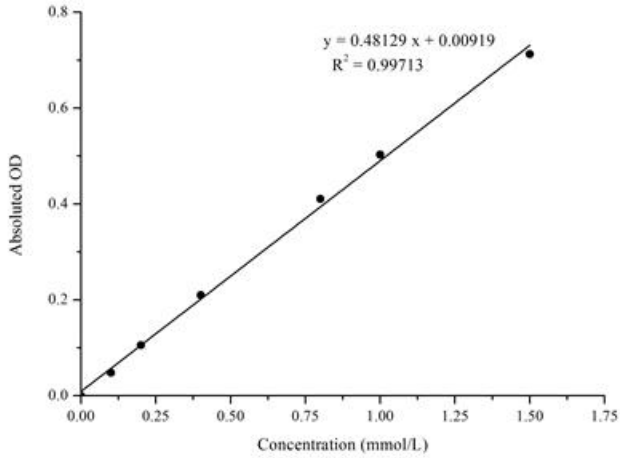
The analytical sensitivity of the assay is 0.01 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

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	1.5
Average OD	0.030	0.078	0.136	0.240	0.441	0.534	0.743
Absoluted OD	0	0.048	0.106	0.210	0.411	0.504	0.713



Appendix Π Example Analysis

Example analysis：

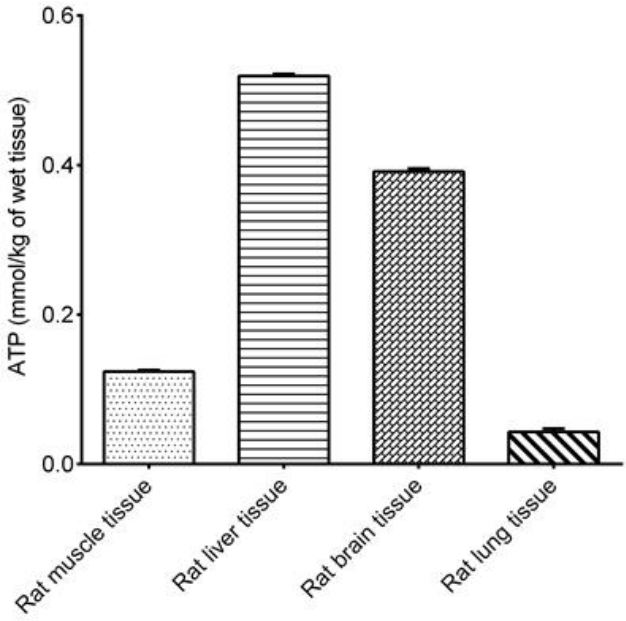
Take rat muscle tissue, treat the sample according to the manual, then dilute the sample with double distilled water for 3 times and carry the assay according to the operation steps. The results are as follows:

The average OD value of the blank is 0.048, the average OD value of the standard is 0.622, the average OD value of the sample is 0.761, the average OD value of the control is 0.758, and the calculation result is:

$$\text{ATP content (mmol/kg wet weight)} = (0.761 - 0.758) \div (0.622 - 0.048) \times 1 \div 0.1 \times 0.9 \times 3$$

$$= 0.14 \text{ mmol/kg wet weight}$$

Detect rat muscle tissue (dilute for 3 times), rat liver tissue (dilute for 2 times), rat brain tissue (dilute for 2 times) and rat lung tissue (dilute for 2 times) according to the protocol, the result is as follows：



Appendix III Publications

1. Fu X Z , Wang Y .Correction to: Interferon-γ regulates immunosuppression in septic mice by promoting the warburg effect through the PI3K/AKT/mTOR pathway[J].Molecular Medicine, 2023, 29(1).DOI:10.1186/s10020-023-00710-w.
2. Malla A , Gupta S , Sur R .Inhibition of lactate dehydrogenase A by diclofenac sodium induces apoptosis in HeLa cells through activation of AMPK[J].FEBS Journal, 2024, 291(16):25.DOI:10.1111/febs.17158.
3. Pan L , Tianjiao E , Xu C ,et al.The apoptotic effects of soybean agglutinin were induced through three different signal pathways by down-regulating cytoskeleton proteins in IPEC-J2 cells[J].Scientific Reports, 2023, 13.DOI:10.1038/s41598-023-32951-4.
4. Sawong S , Pekthong D , Suknoppakit P ,et al.Calotropis gigantea stem bark extracts inhibit liver cancer induced by diethylnitrosamine[J].Scientific reports, 2022, 12(1):12151.DOI:10.1038/s41598-022-16321-0.
5. Olaniyi K S , Areloegbe S E .Acetate ameliorates ovarian mitochondrial dysfunction in letrozole-induced polycystic ovarian syndrome rat model by improving mitofusin-2[J].Journal of Physiological Sciences, 2024, 74(1).DOI:10.1186/s12576-024-00908-5.

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.