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

Catalog No：E-BC-K774-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(450-470 nm)

Detection range：0.72-40.00 μmol/L

Elabscience® ATP 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

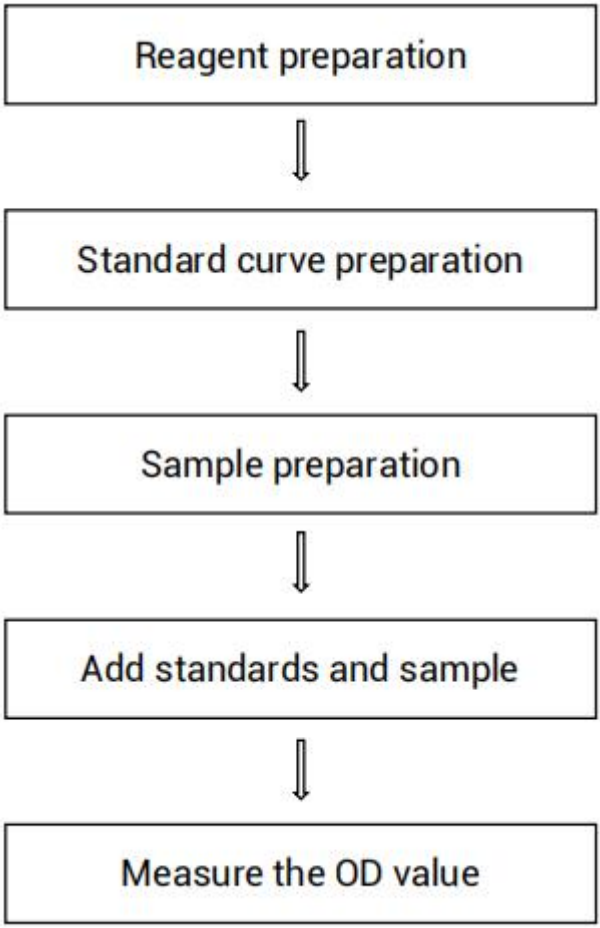
Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

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

Detection principle

Adenosine Triphosphate (ATP) is a high-energy phosphate compound, which is the most direct energy source in the body, and its content is directly related to the state of energy metabolism in the body. Hexokinase (HK) catalyzes the generation of glucose and ATP into glucose-6-phosphate, and glucose-6-phosphate dehydrogenase (G6PD) further catalyzes the dehydrogenation of glucose-6-phosphate into NADPH. NADPH can react with the color developer, and the product has a characteristic absorption peak at 460 nm to determine the ATP content.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Enzyme diluent	8 mL × 1 vial	-20°C, 12 months
Reagent 3	Enzyme Reagent A	Powder × 1 vial	-20°C, 12 months, shading light
Reagent 4	Enzyme Reagent B	0.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Enzyme Reagent C	0.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Chromogenic Agent	6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 7	100 μmol/L Standard Solution	1.5 mL × 2 vials	-20°C, 12 months
	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:

Microplate reader (450-470 nm, optimum wavelength: 460 nm), Incubator (37℃), Water bath

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Keep enzyme reagent A on ice during use. Equilibrate other reagents to 25°C before use.

② The preparation of enzyme A application solution:
Dissolve one vial of enzyme reagent A with 1 mL of double distilled water， mix well to dissolve. Store at -20°C for a month protected from light.

③ The preparation of enzyme A working solution:
Before testing, please prepare sufficient enzyme A working solution according to the test wells. For example, prepare 75 μL of enzyme A working solution (mix well 5 μL of enzyme A application solution and 70 μL of double distilled water). Store at -20°C and used up with the same day.

④ The preparation of enzyme B working solution:
Dissolve one vial of enzyme reagent B with 2.3 mL of enzyme diluent, mix well to dissolve. Store at -20°C for a month protected from light.

⑤ The preparation of enzyme C working solution:
Dissolve one vial of enzyme reagent C with 3.3 mL of enzyme diluent, mix well to dissolve. Store at -20°C for a month protected from light.

⑥ The preparation of 40 μmol/L standard solution:
Before testing, please prepare sufficient 40 μmol/L standard solution.

For example, prepare 1000 μL of 40 μmol/L standard solution (mix well 400 μL of 100 μmol/L standard solution and 600 μL of extraction solution). Store at -20°C for a month.

⑦ The preparation of standard curve:

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

Dilute 40 μmol/L standard solution with extraction solution to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 40 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**40**
40 μmol/L Standard (μL)	0	100	200	300	400	500	600	800
Extraction solution (μL)	800	700	600	500	400	300	200	0

Sample preparation

① Sample preparation

Animal tissue sample:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

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

④ Incubate in boiling water bath for 3 min, cool the tubes to 25°C with running water.

④ Centrifuge at 10000×g for 10 min at 4°C 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% Mouse heart tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse muscle tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Fish muscle tissue homogenate	1

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

The key points of the assay

The fresh samples should be used.

Operating steps

① Standard well: Add 100 μL of different concentrations solution to standard well.

Sample well: Add 100 μL of sample to sample well.

Control well: Add 100 μL of sample to control well.

② Add 50 μL of enzyme A working solution to each well.

③ Add 50 μL of enzyme C working solution to standard and sample wells.

Add 50 μL of enzyme B working solution to control wells.

④ Add 50 μL of chromogenic agent to each well.

⑤ Mix fully with microplate reader for 5 s. Incubated at 37°C for 5 min, measure the OD value of each well at 460 nm with microplate reader.

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:

Tissue sample:

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

[Note]

ΔA：$\Delta A = OD_{sample} - OD_{control}$.

m: The wet weight of sample, g.

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

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 (μmol/L)	7.0	21.0	35.0
%CV	1.5	2.2	2.1

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 (μmol/L)	7.0	21.0	35.0
%CV	6.4	2.8	1.7

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 104.4%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	7.0	21.0	35.0
Observed Conc. (μmol/L)	6.6	23.3	37.9
Recovery rate (%)	94.3	110.8	108.3

Sensitivity

The analytical sensitivity of the assay is 0.72 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

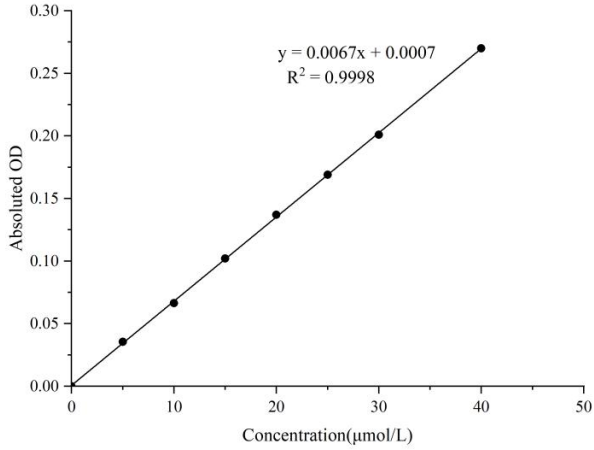
corresponding concentration.

2. Standard curve

(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 (μmol/L)	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**40**
OD value	0.070	0.103	0.135	0.171	0.207	0.238	0.271	0.341
	0.068	0.106	0.136	0.171	0.205	0.238	0.269	0.337
Average OD value	0.069	0.105	0.136	0.171	0.206	0.238	0.270	0.339
Absoluted OD value	0.000	0.036	0.067	0.102	0.137	0.169	0.201	0.270



Appendix Π Example Analysis

Example analysis：

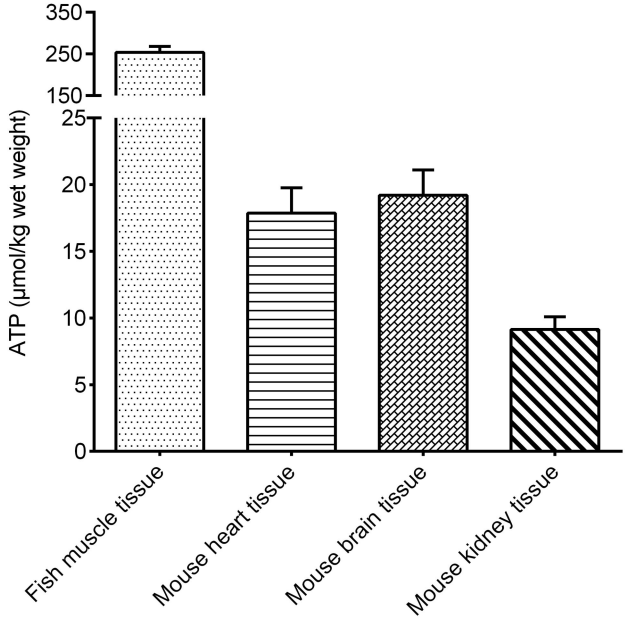
Take 100 μL of 10% fish muscle tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0067 x + 0.0007. the average OD value of the sample well is 0.432, the average OD value of the control well is 0.243, ΔA = 0.432 - 0.243 = 0.189, and the calculation result is:

ATP content (μmol/kg wet weight) = (0.189 - 0.0007) ÷ 0.0067 ÷ 0.1 × 0.9

= 252.94 μmol/kg wet weight

Detect 10% fish muscle tissue homogenate, 10% mouse heart tissue homogenate, 10% mouse brain tissue homogenate and 10% mouse kidney tissue homogenate 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.