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

Catalog No：E-BC-F002

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

Measuring instrument：Chemiluminescence immunoassay analyzer,

Multifunctional microplate reader

Detection range：0.003-5 μmol/L

Elabscience®ATP Chemiluminescence Assay Kit

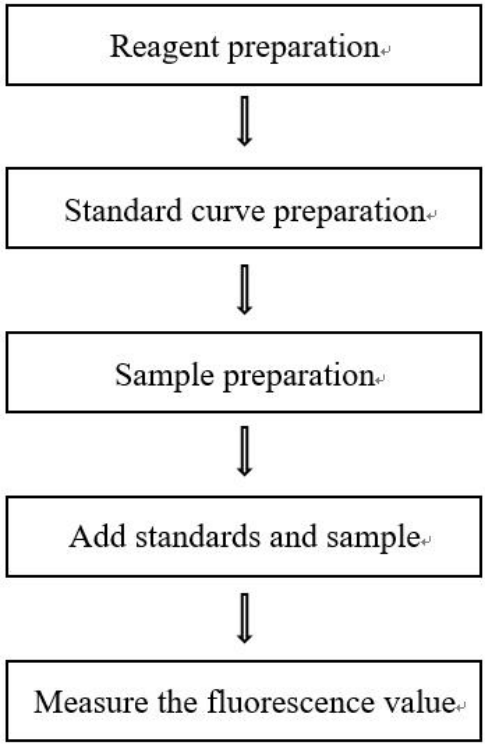
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 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 and cell samples.

Detection principle

Under the catalyzation of luciferase, ATP react with luciferin and emits fluorescence, and the fluorescence intensity is proportional to the concentration of ATP within a certain range.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	50 mL ×1 vial	50 mL × 2 vials	-20℃, 12 months
Reagent 2	100 μmol/L Standard Solution	1 mL ×1 vial	1 mL × 1 vial	-20℃, 12 months
Reagent 3	Enzyme Reagent	Power ×1 vial	Power × 2 vials	-20℃, 12 months shading light
Reagent 4	Enzyme Diluent	7 mL ×1 vial	14 mL × 1 vial	-20℃, 12 months
	Black Microplate	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:

Vortex mixer, Centrifuge, Water bath, Chemiluminescence immunoassay analyzer or multifunctional microplate reader (with the function of detecting chemiluminescence)

Reagent preparation

① Preserve enzyme reagent on ice for use. Equilibrate other reagents to room temperature before use.

② The preparation of enzyme stock solution:

Dissolve one vial of enzyme reagent with 1 mL of enzyme diluent, mix well to dissolve. Store at -20°C for 1 month protected from light.

③ The preparation of enzyme working solution:

Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 120μL of enzyme working solution (20 uL of enzyme stock solution and 100 uL of enzyme diluent). The enzyme working solution should be prepared on spot.

④ 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 extracting solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 2.5, 3, 4, 5 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.5**	**1**	**2**	**2.5**	**3**	**4**	**5**
100 μmol/L standard (μL)	0	5	10	20	25	30	40	50
Extracting solution (μL)	1000	995	990	980	975	970	960	950

Sample preparation

① Sample preparation

Tissue sample:

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

④ Then incubate in boiling water bath for 2 min.

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

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 2×10^6 cells in 300 μL extracting solution with a ultrasonic cell disruptor at 4℃.

④ Then incubate in boiling water bath for 10 min, cool the tubes to room temperature with running water.

⑤ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect su pernatant 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% Mouse lung tissue homogenate	1

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

The key points of the assay

① Dilute the samples to the optimal concentration for detection if the ATP content of samples exceed the detection range.

② The sample size of each batch should be less than 30 (including standard wells).

③ Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

④ It is recommended to aliquot the enzyme reagent working solution into smaller quantities and store at -20℃. Avoid repeated freeze-thaw cycles.

Operating steps

① Standard well: add 100 μL of enzyme working solution into the corresponding well and stand for 5 min.
Sample well: add 100 μL of enzyme working solution into the corresponding well and stand for 5 min.

② Standard well: add 100 μL of standard with different concentrations into standard well, and mix fully immediately.
Sample well: add 100 μL of sample supernatant into sample well, and mix fully immediately.

③ Measure the fluorescence values of each well by the chemiluminescence immunoassay analyzer or multifunctional microplate reader.

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 sample:

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

2. Cell sample:

$$\text{ATP content (μmol/1 × 10^9)} = (\Delta F - b) \div a \times f \div n \times V$$

[Note]

ΔF: The absolute fluorescence value of sample, $F_{Sample} - F_{Blank}$.

f: Dilution factor of sample before tested.

m: wet weight of sample, 0.05 g is recommended.

V: The volume of homogenate medium during the preparation of tissue or cell sample, mL.

n: the number of cells. For example, the number of cells is 5×10^6, N is 5.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse 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)	0.40	2.30	4.00
%CV	3.5	1.6	1.5

Inter-assay Precision

Three mouse 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)	0.40	2.30	4.00
%CV	7.9	6.0	5.6

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 102%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	0.8	2.3	3.7
Observed Conc.(μmol/L)	0.8	2.3	3.9
Recovery rate (%)	101	99	106

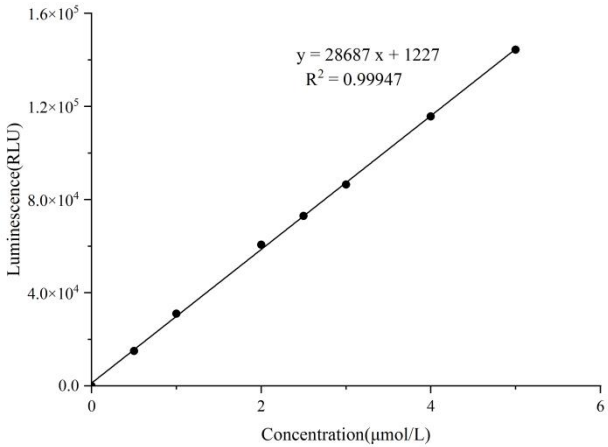
Sensitivity

The analytical sensitivity of the assay is 0.003 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	0.5	1	2	2.5	3	4	5
Fluorescence value	20	15066	31034	60635	72975	86500	115711	144427
	26	15064	31034	60631	72977	86502	115709	144431
Average fluorescence value	23	15065	31034	60633	72976	86501	115710	144429
Absoluted fluorescence value	0	15041	31010	60610	72953	86478	115687	144405



Appendix II Example Analysis

Example analysis:

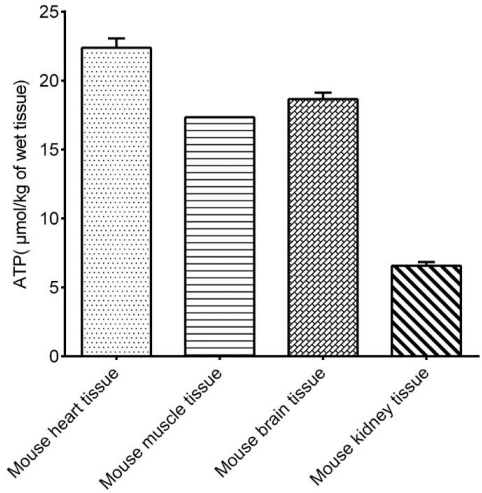
For mouse lung tissue, take 0.05 g of fresh mouse lung sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 26257 x + 1070, the average F value of the sample is 19512, the average F value of the blank is 92, and the calculation result is:

$$\text{ATP content (μmol/kg wet tissue)} = (19512\text{-}92\text{-}1070) \div 26257 \div 0.05 \times 0.45$$

$$= 6.29\ \text{μmol/kg wet weight}$$

Detect 10% mouse heart tissue homogenate, 10% mouse muscle 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.