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

Catalog No：E-BC-F201

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

Measuring instrument：Chemiluminescence immunoassay analyzer

Detection range：0.002-4 μmol/L

Elabscience® Enhanced 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

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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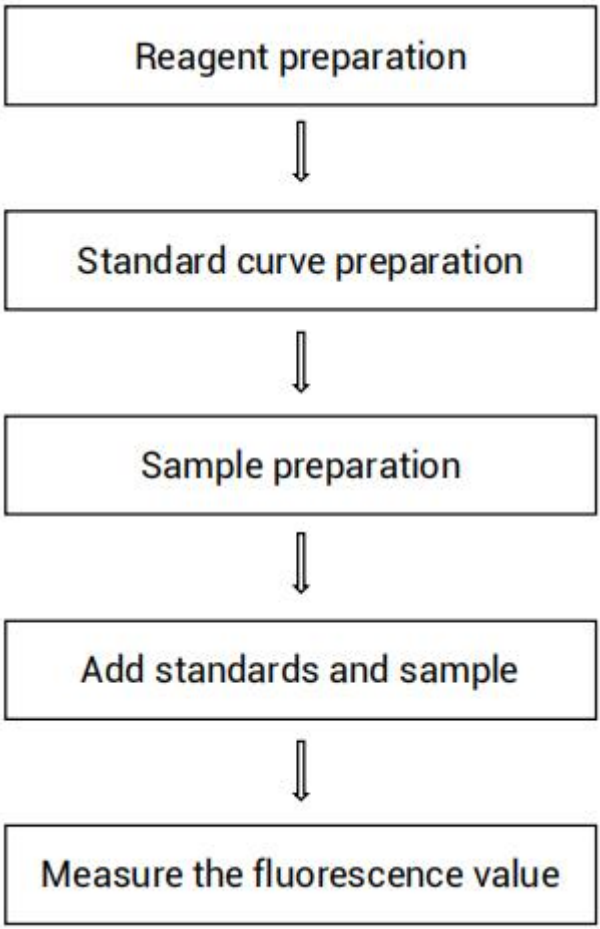
Assay summary



Intended use

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

Detection principle

Luciferase combines with luciferin and undergoes an oxidation reaction in the presence of ATP and oxygen, releasing fluorescence. When both luciferase and luciferin are in excess, within a certain range, the fluuorescence intensity of the light signal is proportional to the ATP content.

Kit components & storage

Item	Component	Size (48 T)	Size (96 T)	Storage
Reagent 1	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 2	Buffer Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months
Reagent 3	100 μmol/L Standard Solution	1 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months
	Black 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:
Chemiluminescence immunoassay analyzer or multifunctional microplate reader; Water bath

Reagent preparation

① Preserve enzyme reagent and buffer solution on ice for use.
Equilibrate other reagents to 25°C before use.

② The preparation of enzyme stock solution:
Dissolve one vial of enzyme reagent with 1 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 1 week protected from light.

③ The preparation of working solution:
Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 240 μL of working solution (20 μL of enzyme stock solution and 220 μL of buffer solution). The working solution should be prepared on spot and used up within 8 h.

④ The preparation of 4 μmol/L standard solution:
Before testing, please prepare sufficient 4 μmol/L standard solution. For example, prepare 625 μL of 4 μmol/L standard solution (25 μL of 100 μmol/L standard solution and 600 μL of buffer solution). The 4 μmol/L standard solution should be prepared on spot and used up within 8 h.

⑤ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 4 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.3, 0.5, 1, 2,3, 4 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.1**	**0.3**	**0.5**	**1**	**2**	**3**	**4**
4 μmol/L standard (μL)	0	5	15	25	50	100	150	200
Buffer Solution (μL)	200	195	185	175	150	100	50	0

Sample preparation

① Sample preparation

Tissue samples:

① Pre-cool buffer solution to 2-8°C. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

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

③ Incubate in boiling water bath for 3 min then cool it down to room temperature (25°C) with running water. Centrifuge at 10000 × g for 5 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The ATP in the homogenized samples is unstable, it is recommended to measure it within 1 h.

Cell (adherent or suspension) samples:

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

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

③ Homogenize 1×10^6 cells in 200 μL Buffer Solution with a ultrasonic cell disruptor at 4˚C.

④ Then incubate in boiling water bath for 5 minutes, cool the tubes to room temperature with Flowing water.

⑤ Centrifuge at 10000×g for 10 minutes 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 spleen tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse muscle tissue homogenate	2-4

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

The key points of the assay

In each experiment, the number of detection wells (including the standard wells) should be within 30.

Operating steps

① Standard well: add 2 μL of standard with different concentrations into the well.

Sample well: add 2 μL of sample into the well.

② Add 200 μL of working solution into each well.

③ Measure the luminescence values of each well by the chemiluminescence immunoassay analyzer or multifunctional microplate reader, as L.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean luminescence value of the blank (Standard #①) from all standard readings. This is the absoluted luminescence value.
3. Plot the standard curve by using absoluted luminescence 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 samples:

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

Cell samples:

$$\frac{\text{ATP content}}{(\mu\text{mol}/1 \times 10\text{^}9)} = \frac{\Delta L - b}{a} \times f \div n \times V$$

[Note]

ΔL: The luminescence values of sample well - luminescence values of blank well

f: Dilution factor of sample before tested.

m: The weight of sample, g

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 10% mouse heart tissue homogenate 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)	1.0	2.0	3.0
%CV	1.8	1.3	1.2

Inter-assay Precision

Three 10% mouse heart tissue homogenate were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.5	2.5	3.5
%CV	1.1	2.4	2.4

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 105.7%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	1.0	2.0	3.0
Observed Conc.(μmol/L)	1.04	2.13	3.17
Recovery rate (%)	104	107	106

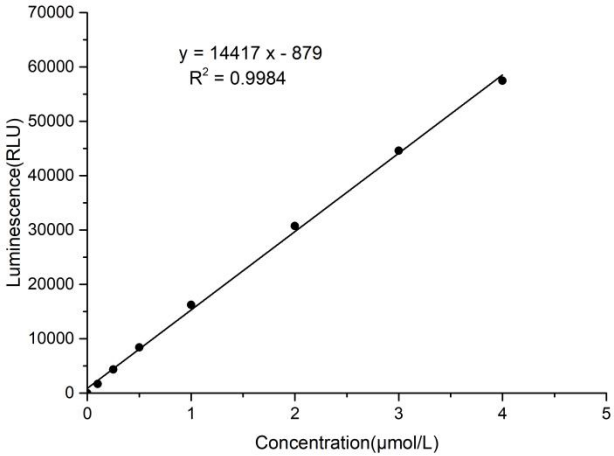
Sensitivity

The analytical sensitivity of the assay is 0.002 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 luminescence 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	0.1	0.3	0.5	1	2	3	4
Luminescence value	134	1812	4428	8653	16535	31283	45291	57034
	145	1870	4546	8464	16197	30447	44154	58142
Average luminescence value	140	1841	4487	8559	16366	30865	44723	57588
Absoluted luminescence value	0	1702	4348	8419	16227	30726	44583	57449



Appendix Π Example Analysis

Example analysis:

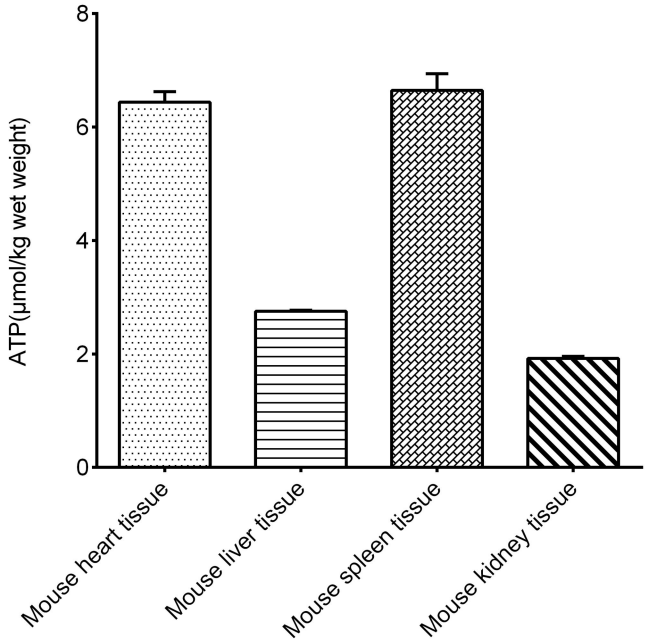
Take 2 μL of 10% mouse heart tissue supernatant and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 13051 x + 819, the average luminescence value of the blank well is 130, the average luminescence value of the sample well is 10289, and the calculation result is:

ATP content (μmol/kg wet weight) = (10289 - 130 - 819) ÷ 13051 ÷ 0.05 × 0.45

= 6.44 μmol/kg wet weight

Detect mouse serum, 10% mouse heart tissue homogenate, 10% mouse liver tissue homogenate, 10% mouse spleen tissue homogenate, 10% mouse kindey 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.