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

Catalog No：E-BC-F300

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

Measuring instrument：Chemiluminescence immunoassay analyzer

Detection range：0.002-1 μmol/L

Elabscience® ATP Chemiluminescence Assay Kit (Double Reagent)

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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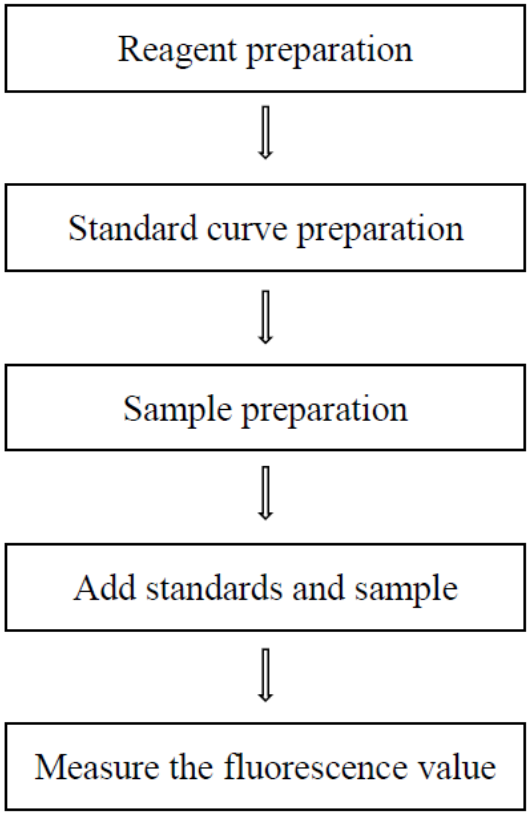
Assay summary



Intended use

This kit can be used to measure the ATP content in 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 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	Enzyme Diluent	7 mL × 1 vial	14 mL × 1 vial	-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		

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

Reagent:

PBS(0.01 M，pH 7.4)

Reagent preparation

① Preserve enzyme reagent 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 enzyme diluent, 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 120 μL of working solution (20 μL of enzyme stock solution and 100 μL of enzyme diluent). The working solution should be prepared on spot and used up within 8 h.

④ The preparation of 1 μmol/L standard solution:

Before testing, please prepare sufficient 1 μmol/L standard solution according to the test wells. For example, prepare 3000 μL of 1 μmol/L standard solution (30 μL of 100 μmol/L standard solution and 2970 μL of PBS(0.01 M，pH 7.4)). The 1 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 1 μmol/L standard solution with PBS(0.01 M，pH 7.4) to a serial concentration. The recommended dilution gradient is as follows: 0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.025**	**0.05**	**0.1**	**0.2**	**0.4**	**0.8**	**1.0**
1 μmol/L standard (μL)	0	25	50	100	200	400	800	1000
PBS (μL)	1000	975	950	900	800	600	200	0

Sample preparation

Cell samples:

① Centrifuge at 300 × g for 5 min at 4℃ to discarded the medium and collect the cell precipitate.

② Add PBS (0.01 M, pH 7.4) and wash the cells by gentle pipetting.

③ Centrifuge at 300 × g for 5 min at 4℃. Discarded the supernatant and collect the cell precipitate.

④ Resuspend the cells in PBS (0.01 M, pH 7.4) to a concentration of 1×10^5/mL.

② Dilution of sample

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

Sample type	Dilution factor
1×10^4 Jurkat cells	1
1×10^4 RAW 264.7 cells	1
1×10^4 HL-60 cells	1
1×10^4 Hela cells	1

Note: The diluent is PBS (0.01 M, pH 7.4). 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 100 μL of standard with different concentrations into the well.

Sample well: add 100 μL of cell suspension into the well.

② Add 100 μL of working solution into each well.

③ Measure the c 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:

Cell samples:

$$\frac{\text{ATP centent}}{(\mu mol/1\times10^{8})} = (\Delta L - b) \div a \div n$$

[Note]

ΔL: The luminescence values of sample well – the luminescence values of blank well.

n: The concentration of cells, 1×10^8/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 1×10^4 Hela cells 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)	0.25	0.50	0.75
%CV	2.5	1.7	2.6

Inter-assay Precision

Three 1×10^4 Hela cells were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	0.25	0.50	0.75
%CV	4.8	3.4	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 97.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc.(μmol/L)	0.25	0.50	0.75
Observed Conc.(μmol/L)	0.25	0.49	0.72
Recovery rate (%)	99	98	96

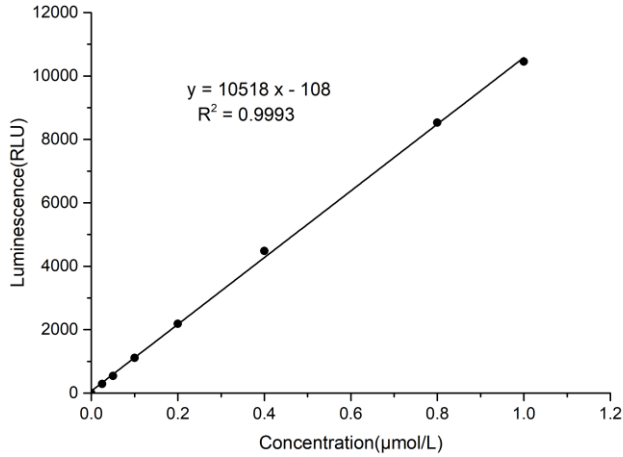
Sensitivity

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 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	0.025	0.05	0.1	0.2	0.4	0.8	1.0
Luminescence value	88	380	625	1220	2237	4563	8593	10540
	80	371	628	1175	2304	4571	8642	10542
Average luminescence value	84	376	627	1198	2271	4567	8618	10541
Absoluted luminescence value	0	292	543	1114	2187	4483	8534	10457



Appendix Π Example Analysis

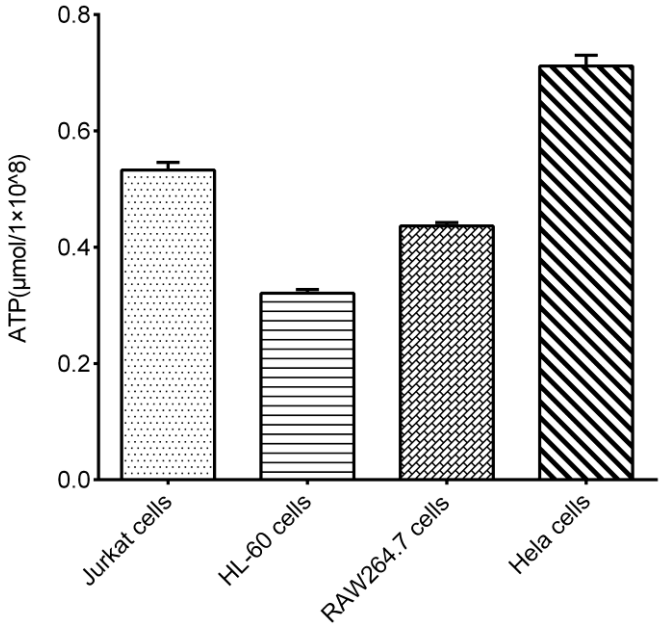
Example analysis：

Take 100 μL of 1×10^5/mL Jurkat cells and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 10420 x + 93, the average luminescence value of the blank well is 28, the average luminescence value of the sample well is 5645, and the calculation result is:

ATP content (μmol/1×10^8) = (5645 - 28 - 93) ÷ 10420 ÷1 = 0.53 μmol/1×10^8

Detect 1×10^5/mL Jurkat cells, 1×10^5/mL HL-60 cells, 1×10^5/mL RAW264.7 cells, 1×10^5/mL Hela cells 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.