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

**Catalog No：E-BC-F004**

**Specification：48T(48 samples)/96T(96 samples)**

**Measuring instrument：Chemiluminescence immunoassay analyzer**

# **Elabscience®** ATP/ADP Ratio 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：

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

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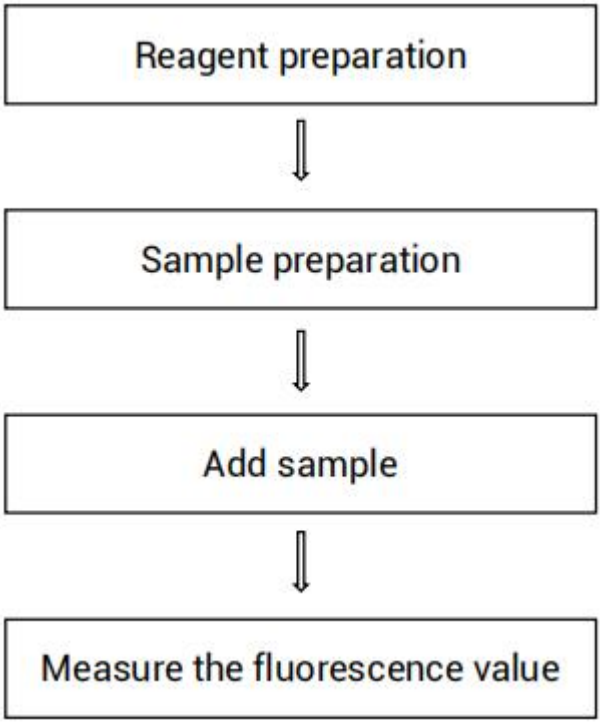
**Assay summary**



## Intended use

This kit can be used to detect the ratio of ATP to ADP in cell samples to analyze cell status.

## Detection principle

Adenosine triphosphate (ATP) is the energy currency of organisms, produced by glycolysis or the tricarboxylic acid cycle and electron transport chain in mitochondria, and is the source of energy in cells. Under normal circumstances, ATP and adenosine diphosphate (ADP) will convert to each other, and the change of the ratio of ATP to ADP is closely related to apoptosis, autophagy, energy metabolism and other pathways, so it is often used as an indicator of cell activity.

Under the catalysis of luciferase, ATP react with luciferin and emits fluorescence. Convert ADP to ATP by enzyme, and ATP was detected by the same principle. The ATP/ADP ratio in the cell can be calculated.

## Kit components & storage

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|---|---|---|---|---|
| Reagent 1 | Buffer Solution | 20 mL × 1 vial | 40 mL × 1 vial | -20℃, 12 months |
| Reagent 2 | Enzyme | Power × 1 vial | Power × 2 vials | -20℃, 12 months shading light |
| Reagent 3 | Enzyme Diluent | 7 mL × 1 vial | 14 mL × 1 vial | -20℃, 12 months |
| Reagent 4 | Substrate | Power × 2 vials | Power × 4 vials | -20℃, 12 months shading light |
| Reagent 5 | Accelerant | 0.5 mL ×1 vial | 1 mL ×1 vial | -20℃, 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 (with the function of detecting luminescence)、incubator

## Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of enzyme stock solution:
Dissolve one vial of enzyme with 1 mL of enzyme diluent, mix well to dissolve. Aliquoted storage at -20°C for 1 month.

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 μL of enzyme stock solution and 100 μL of enzyme diluent). The enzyme working solution should be prepared on spot.

④ The preparation of reaction working solution:
Dissolve one vial of substrate with 1.49 mL of buffer solution, then add 10 μL of accelerant, mix well to dissolve. The reaction working solution should be prepared on spot.

## Sample preparation

**Sample preparation**

**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 Add 150 μL buffer solution to 1×10^6 cells and mix fully.

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.

## The key points of the assay

① It is recommended to aliquot the enzyme stock solution into smaller quantities and store at -20℃. Avoid repeated freeze-thaw cycles.

② The sample size of each batch should be less than 30.

## Operating steps

① Sample well: add 100 μL of enzyme working solution into the corresponding well and stand for 5 min at 25℃. Measure the luminescence values of each well by the chemiluminescence immunoassay analyzer, as $L_1$.

② Sample well: add 20 μL of sample supernatant into sample wells, and mix fully with chemiluminescence immunoassay analyzer for 5 s. Measure the luminescence values of each well, as $L_2$.

③ Stand for 30 min at 25℃, measure the luminescence values of each well by the chemiluminescence immunoassay analyzer, as $L_3$.

④ Sample well: add 50 μL of reaction working solution into each well and stand for 6 min at 25℃.

③ Mix fully with chemiluminescence immunoassay analyzer for 5 s. Measure the luminescence values of each well, as $L_4$.

## Calculation

$$\text{ATP/ADP} = \frac{L_2 - L_1}{L_4 - L_3}$$

**[Note]**

$L_1$: The luminescence values of black microplate.

$L_2$: The luminescence values of system after adding the sample (The luminescence values of ATP = $L_2 - L_1$).

$L_3$: The luminescence values of system before adding ADP convertase.

$L_4$: The luminescence values of system after adding ADP convertase (The luminescence values of ADP = $L_4 - L_3$).

## 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.