Enhanced ATP Assay Kit

A1508155

Storage: -20℃ (For detailed storage conditions, please refer to the Kit Components).
Shipping: Shipped with ice packs. Upon receipt, please promptly store the product under the recommended storage conditions.

Product Introduction

Adenosine triphosphate (ATP) is the chemical energy source for cellular metabolism and is commonly known as the "energy currency" of cells. ATP is produced in living cells through photosynthesis and cellular respiration, and provides energy for a variety of vital life processes, including biosynthesis, cell motility, and cell division. As a core indicator reflecting cellular activity, ATP is widely used in scientific research and drug development to evaluate cell viability and cytotoxicity. Typically, intracellular ATP levels decline during cell apoptosis, necrosis, or exposure to toxic conditions; by contrast, stimuli such as high glucose can upregulate intracellular ATP content in certain cell types.

This kit is developed based on the principle that firefly luciferase requires ATP to provide energy for catalyzing the luminescent reaction of D-luciferin. When firefly luciferase and luciferin are present in excess, the luminescence intensity is positively correlated with ATP concentration within a certain linear range, enabling highly sensitive detection of ATP levels in samples.

Kit Components

A1508155	Components	Appearance	100T	10×100T	Storage	Volume Per Test
A1508155A	5×ATP Detection Lysis Buffer	Colorless clear liquid	20 mL	10×20 mL	-20℃	On request
A1508155B	10×ATP Assay Buffer	Colorless clear liquid	10 mL	10×10 mL	-20℃	100 μL
A1508155C	D-luciferin	Yellow liquid	0.1 mL	10×0.1 mL	-20℃. Protect from light	On request
A1508155D	Luciferase	Colorless clear liquid	0.2 mL	10×0.2 mL	-20℃	On request
A1508155E	Cofactor	Colorless clear liquid	0.1 mL	10×0.1 mL	-20℃	On request
A1508155F	ATP Standard (500μM)	Colorless clear liquid	0.1 mL	10×0.1 mL	-20℃	On request

Procedure (All procedures should be performed at 4℃)

1. Additional Required Materials

(1) Self-provided reagent: PBS

(2) Required instrument: Multifunctional microplate reader (equipped with chemiluminescence detection function).

(3) Others: Opaque white or black 96-well plate, low-speed refrigerated centrifuge, homogenizer (for tissue samples).

2. Solution Preparation

(1) Preparation of 1**×ATP Lysis Buffer** Dilute the 5×ATP Detection Lysis Buffer with deionized water at a ratio of 4:1 for later use. Example: Add 4 mL of deionized water to 1mL of 5×ATP Detection Lysis Buffer and mix well to prepare 1×ATP Lysis Buffer.

(2) **Cofactor**: Ready-to-use. Aliquot the solution into 10 equal portions of 10 μL each before use, and store frozen at ≤ -20℃.Thawed aliquots should be kept on ice or at 4℃ until use.

(3) Preparation of **1×ATP Assay Buffer**: Dilute the 10×ATP Assay Buffer with deionized water at a ratio of 9:1 for later use. Example: Add 9 mL of deionized water to 1 mL of 10× ATP Assay Buffer and mix well to prepare 1×ATP Assay Buffer.

(4) **D-luciferin**: Ready-to-use. Aliquot into 10 portions immediately before use, store frozen at ≤ -20℃ and protected from light until needed.

(5) Prepare an appropriate volume of **ATP Working Solution** according to the number of test samples with reference to the table below. Mix the related reagents in the specified proportion to obtain the final ATP Working Solution. If the prepared working solution cannot be used immediately, store it under light-protected conditions.

Component	1 Sample	10 Sample	100 Sample
1×ATP Assay Buffer	96 μL	960 μL	9600 μL
D-luciferin	1 μL	10 μL	100 μL
Luciferase	2 μL	20 μL	200 μL
Cofactor	1 μL	10 μL	100 μL

Note: Unused reagents should be aliquot and stored at -20℃ protected from light to avoid repeated freeze-thaw cycles.

3. Sample Preparation (Note: Perform all lysis steps at 4℃ or on ice**)**

(1) **Adherent cells**: Aspirate the culture medium. Add 1×ATP Lysis Buffer at a ratio of 200 μL per well of a 6-well plate (equivalent to 1/10 of the 2 mL culture medium volume). To ensure complete lysis, repeatedly pipette the lysate or rock the plate to ensure the 1×ATP Lysis Buffer fully contacts the cells. Cells typically lyse immediately upon contact with the 1×ATP Lysis Buffer. After lysis, centrifuge at 12,000×g for 5 minutes at 4℃, then collect the supernatant for subsequent assays.

(2) **Suspension cells**: Pellet the cells by centrifugation, discard the supernatant, and gently resuspend the cell pellet. Add 1×ATP Lysis Buffer at a ratio of 200 μL per well-equivalent

cell count (as in a 6-well plate). To ensure complete lysis, tap the bottom of the centrifuge tube or vortex briefly to ensure the 1×ATP Lysis Buffer fully contacts the cells. Cells typically lyse immediately upon contact with the 1×ATP Lysis Buffer. After lysis, centrifuge at 12,000×g for 5 minutes at 4℃, then collect the supernatant for subsequent assays.

(3) **Tissue samples**: Add 1×ATP Lysis Buffer at a ratio of approximately 100-200 μL per 20 mg of tissue, then homogenize using a glass homogenizer or other homogenization equipment. Thorough homogenization ensures complete tissue lysis. After lysis, centrifuge at 12,000×g for 5 minutes at 4℃, then collect the supernatant for subsequent assays.

4. Preparation for Standard Curve Assay

Thaw all reagents on ice. Dilute the ATP Standard (500μM) with 1×ATP Lysis Buffer to prepare appropriate concentration gradients. The exact concentrations should be determined based on the expected ATP concentration in the samples. For initial testing, concentrations of 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μM are recommended. In subsequent experiments, the range of standard concentrations can be adjusted according to the ATP levels in the samples.

Tube ID	1×ATP Lysis Buffer Volume (μL)	ATP Standard (500μM) Volume	Final Concentration (μM)
A	98	2 μL from ATP Standard (500μM)	10
B	70	30 μL from Tube A	3
C	90	10 μL from Tube A	1
D	90	10 μL from Tube B	0.3
E	90	10 μL from Tube C	0.1
F	90	10 μL from Tube D	0.03
G	90	10 μL from Tube E	0.01

5. ATP Concentration Assay

(1) Add 100 μL of **ATP Working Solution** to each assay well or tube.

(2) Add 20 μL of sample or standard to each well/tube, mix gently, and incubate at 37℃ for 15-20 minutes. After the reaction is complete, measure the RLU (Relative Light Unit) using a multifunctional microplate reader.

Notes:

① The sample volume can be adjusted within the range of 10-100 μL as needed. For samples with low ATP levels, use 100 μL of sample; for high ATP levels, use a smaller volume of sample. The same volume must be used for standards and samples. If the ATP concentration in the sample is extremely high, dilute the sample with 1×ATP Lysis Buffer prior to measurement. When using 10-100 μL of standard, the kit exhibits excellent linearity in the range of 0.1 nM to 10 μM.

② For high-concentration ATP samples, the incubation time may be appropriately extended (no more than 30 minutes) to ensure the reaction reaches a stable plateau before reading

and achieve optimal linear correlation; For low-concentration ATP samples, the luminescent signal increases rapidly under standard incubation conditions. A stable and detectable signal is generally achieved within 3-5 minutes, and the measurement can be taken in advance to improve experimental efficiency.

Precautions

1. This kit is for research use only. It shall not be used for clinical diagnosis or any other purposes. It is prohibited for food or pharmaceutical applications, and the kit shall not be stored in residential premises.
2. Read the user manual carefully and calibrate all instruments before the experiment. Perform the assay strictly in accordance with the instructions.
3. Wear a lab coat and latex gloves for personal protection during the experiment.
4. The ATP detection reagent contains firefly luciferase, which will gradually lose activity upon repeated freeze-thaw cycles. For optimal performance, aliquot the reagent properly after the first thawing; ensure all aliquot containers are free of ATP contamination. Once diluted into working solution, the ATP detection reagent is recommended for single use only and should not be refrozen for subsequent use.
5. ATP, especially ATP in lysed samples, is unstable at room temperature. All operations should be performed at 4℃ or on ice.
6. A luminometer (the instrument commonly used for firefly luciferase reporter gene detection) is required for this assay. A liquid scintillation counter may be used as an alternative; its detection performance depends on the instrument's sensitivity and precision.
7. When using a multifunctional microplate reader with chemiluminescence detection function, use white or black 96-well plates with light-tight gaps between wells. If standard transparent 96-well plates are adopted, arrange blank spacing wells between sample wells to eliminate cross-well interference.
8. Run standard samples in parallel with each experiment. The background luminescence of working solution increases over time, which will reduce assay sensitivity.
9. The luciferin-luciferase reaction is extremely sensitive; avoid exogenous biological ATP contamination derived from bacteria, fingerprints and other biological sources. Use ATP-free ultrapure water, pipette tips, centrifuge tubes and other consumables throughout the experiment.
10. Luciferin and luminescent reaction buffer are light-sensitive. Light exposure accelerates reagent inactivation and elevates background signals. Keep all operations protected from light.
11. Mix solutions containing firefly luciferase gently by inverting the tube. Do not vortex, as vigorous shaking may cause enzyme denaturation.
12. Arsenate compounds can inhibit this reaction system. In addition, high salt concentration in samples commonly suppresses luciferase activity and reduces detection sensitivity.