Luminescent ATP Cell Viability Assay Kit I

Introduction

ATP is an important source of energy within cells and can be used to measure the level of metabolism in cells. ATP levels are also positively correlated with the number of viable cells. Therefore, ATP levels can be used to measure the number of viable cells.

Luminescent ATP Cell Viability Assay Kit I is a kit that uses firefly luciferase to detect cell viability. The reagent provided in this kit contains thermal-stable firefly luciferase and a highly purified substrate luciferin. This reagent can lyse cells and release ATP, which then produces a stable luminescence signal catalyzed by luciferase. The intensity of its luminescence signal is proportional to the number of viable cells.

The reagent provided in this kit is a ready-to-use solution and does not require cell lysis in advance, making it very convenient to use. Compared to other cell viability reagents, such as MTT, CCK-8, Alamar Blue, and Calcein-AM, this reagent offers higher sensitivity and shorter detection times. In addition, the reagent has good linearity in 10-30,000 cells. This kit has been tested to be as effective as CellTiter-Glo® Luminescent Cell Viability Assay (CTG).

Components and Storage

Components	K2041-100 T	K2041-1000 T
Luminescent ATP Cell Viability Assay Reagent	10 mL	100 mL
Store the reagent at -80°C away from light, stable for at least 1 year. Or store the reagent at -20°C away from light, stable for 6 months.		

Protocol

1. **Cell seeding:** Use a plate suitable for chemiluminescent detection for cell seeding, such as a white or black opaque culture plate. For 96-well plates, add 100 μL culture medium containing cells per well (< 30,000 cells/well at the time of detection), and for 384-well plates, add 25 μL of culture medium containing cells per well (< 6,000 cells/well at the time of detection). At the same time, a blank control group (without cells, medium only) needs to be set up. Cells are cultured as usual.

***Note:** The optimal cell number for seeding can be adjusted according to the size of the plate.

2. **Drug treatment (optional):** If desired, treat cells with the drug of interest for a period depending on the

experimental design. In this case, it is recommended that in addition to the blank control, a negative control group (containing cells, culture medium, and drug solvent) should be set up.

3. **Detection reagent preparation:** Thaw the Luminescent ATP Cell Viability Assay Reagent at 4°C or room temperature in advance, or melt the reagent in a 22°C water bath, noting that the water bath temperature should not exceed 25°C. Gently invert several times before use to mix the reagent well.

***Note:** Luciferase activity is temperature sensitive. Therefore, this reagent needs to be equilibrated to room temperature before use.

4. **Detection:**

1) Remove the plate from the incubator and equilibrate at room temperature for 10 min.

***Note:** It is recommended that the equilibration time should not exceed 30 min.

2) Add Luminescent ATP Cell Viability Assay Reagent equal to the volume of culture medium in each well. For a 96-well plate, add 100 µL Luminescent ATP Cell Viability Assay Reagent to 100 µL culture medium in each well.

3) Shake the plate for 2 minutes at room temperature to induce cell lysis.

4) Incubate at room temperature for 10 minutes to stabilize the luminescence signal.

5) Record the luminescence signal RLU using an instrument with a chemiluminescence detection module, such as a multimode microplate reader. The corresponding parameters can be set according to the requirements of the instrument, and the integration time can generally be set to 0.25-1 s per well.

6) Calculate the relative viability of the cells directly using the following formula, or calculate the ATP content from the ATP standard curve and then calculate the relative viability of the cells.:

$$\text{Cell viability (\%)} = [(RLU_{\text{Experimental group}} - RLU_{\text{Blank control group}})/(RLU_{\text{Negative control group}} - RLU_{\text{Blank control group}})] \times 100\%$$

***Note:** The results may vary depending on the cell type or status, and for some cells with particularly high ATP content, there may not be a significant linear relationship when the number of cells reaches 30,000, but the luminescence reading will continue to increase.

5. **ATP Standard Curve (optional):**

1) Dilute the ATP standard solution with cell culture medium into a series of appropriate concentration gradients. 0, 0.1, 0.5, 1, 2, 4, 6, 8, 10 µM series concentrations can be set for the initial assay.

***Note:** This kit does not provide the ATP standard solution.

2) Add the series of concentration gradients of ATP standard in a 96-well plate in 100 µL. Then add Luminescent ATP Cell Viability Assay Reagent equal to the volume of ATP standard in each well.

***Note:** When diluting ATP standards with medium, they need to be tested immediately after dilution, as enzymes in serum (e.g., ATPase) can deplete ATP.

3) Shake the plate for 2 minutes at room temperature to induce cell lysis.

4) Incubate at room temperature for 10 minutes to stabilize the luminescence signal.

5) Record the luminescence signal RLU using an instrument with a chemiluminescence detection module, such as a multimode microplate reader. The corresponding parameters can be set according to the requirements of the instrument, and the integration time can generally be set to 0.25-1 s per well.

***Note:** Establishing an ATP standard curve is an optional step, e.g., an ATP standard curve may not be created when measuring cell viability.

Note

1. When this kit is stored at -20°C, its detection effect will gradually decrease, so it is recommended to store it at -80°C in the dark. Alternatively, you can choose the Luminescent ATP Cell Viability Assay Kit II (Catalog. Number: K2402), which can be stored at -20°C for long periods.
2. The reagent in this kit contains luciferase, so repeated freeze-thaw cycles need to be avoided as much as possible. After testing, repeatedly frozen and thawed 5 times has no significant effect on its detection effect. In addition, it is not recommended to aliquot this reagent because of the potential for ATP contamination. If an aliquot is needed, the container must not be contaminated with ATP.
3. A small amount of precipitate may appear in this reagent after repeated freeze-thaw cycles, which can be dissolved when warmed to room temperature. If there is still a small amount of precipitate at room temperature, it can be removed by centrifugation and used without affecting the detection effect.
4. When handling drugs, high levels of drug solvents in the system may affect the enzymatic reaction. It is recommended to set up a blank control (containing cells, media, and drug solvent) to rule out solvent interference. In general, the use of less than 2% DMSO, methanol, or ethanol in the system will not affect the test results.
5. It is recommended to use white or black opaque cell culture plates to seed cells to avoid interference between adjacent wells during detection.
6. For your safety and health, please wear lab coats and gloves during the experiment.
7. For research use only. Not to be used in clinical diagnostic or clinical trials.

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