ATP Luminescent Cell Viability Assay Kit

Cat. No. KITG-032 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	
Description	ATP is the most important energy molecule within cells and can be used to measure cellular metabolic activity. ATP content exhibits a good linear relationship with the number of viable cells, making it an excellent indicator of cell viability. The principle of ATP bioluminescence technology relies on the luciferase enzyme, which utilizes luciferin, adenosine triphosphate (ATP), and O_2 as substrates. In the presence of Mg^{2+}, luciferase converts chemical energy into light energy. ATP serves as both the essential substrate for the luciferase-catalyzed luminescence reaction and the energy source for all biological life activities. Within a certain concentration range, ATP concentration is linearly proportional to luminescence intensity. This assay kit employs a bioluminescent method using Firefly luciferase to catalyze the conversion of the substrate luciferin, efficiently utilizing ATP energy to emit photons. The luminescence signal is directly proportional to the amount of ATP present, which is directly proportional to the number of cells in the culture medium. The luminescent cell viability detection reagent provided in this kit offers a broad linear range, high sensitivity, and excellent stability. In a 96-well plate, a good linear relationship is observed within the range of 5 to 100,000 cells per well, though the upper detection limit may vary depending on the cell type. Additionally, the procedure is simple and straightforward; the detection reagent provided in the kit is ready-to-use, and the readout is stable with rapid detection speed, requiring only approximately 10 minutes to complete the assay. There is no need to wash cells, change medium, or remove culture supernatant. The reagent also has a long half-life, providing excellent stability.

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Form	Luminescent Cell Viability Assay Kit
Applications	Measurement of cellular metabolic activity and cell viability through ATP bioluminescence detection. Suitable for 96-well and 384-well plate formats. For research use only.
Notes	Assay Protocol: 1. Cell Culture: Use a 96-well plate suitable for chemiluminescence detection. Seed 100 µ of cell suspension per well (adjust initial cell density based on culture duration; do not exceed 50,000 cells per well at detection). Include a negative control well containing medium without cells. Incubate cells at 37°C with 5% CO_2. A cell concentration gradient may be prepared to obtain optimal experimental results. Treat cells with test compounds at the desired time points as needed. 2. (Optional) Preparation of ATP Standard Curve: Dilute the ATP standard solution with PBS to prepare an appropriate concentration gradient. Add 100 µL of each standard to the 96-well plate. 3. Cell Viability Detection: 1) Thaw the luminescent detection reagent and equilibrate to room temperature (do not thaw above 25°C to avoid activity loss). 2) Remove the cell culture plate and allow it to equilibrate to room temperature for 10–20 minutes. 3) Add 100 µL of detection reagent per well of a 96-well plate (25 µL per well for a 384-well plate). Note: Due to edge effects, it is not recommended to plate cells in the outermost wells. 4) Shake the plate at room temperature for 2–5 minutes to promote cell lysis. 5) Seal the plate with an opaque sealing film and incubate at room temperature in the dark for 10 minutes to allow the luminescence signal to reach maximum intensity. 6) Measure chemiluminescence using a multimode microplate reader. Set instrument parameters according to the manufacturer's instructions. Typical detection time per well is 0.25–1 second; adjust based on instrument sensitivity. 7) Calculate relative cell viability based on chemiluminescence readings, or determine ATP content using the ATP standard curve to derive relative cell viability. Note: Detection performance varies among different cell types. For

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	certain cells with particularly high ATP content, chemiluminescence readings may continue to increase when cell numbers exceed 100,000, but the linear relationship will be lost. Precautions: 1. Luciferase activity is temperature-sensitive. Both cells and detection reagent must be equilibrated to room temperature before the assay. Do not store the reagent at room temperature. 2. Mix the detection reagent thoroughly before use. 3. The detection reagent in this kit contains luciferase, which will gradually lose activity upon repeated freeze-thaw cycles. For optimal performance, aliquot the reagent after the first thaw. Ensure that the aliquoting containers are free of ATP contamination. 4. High solvent concentrations in test compounds may interfere with the luciferase reaction and affect the chemiluminescence signal. This interference can be excluded by including a control well containing the solvent in cell culture medium. 5. Use white or black 96-well or 384-well plates suitable for cell culture. Transparent plates will cause cross-talk between adjacent wells. 6. For your safety and health, wear a lab coat and disposable gloves during operation. 7. For research use only.
Storage	Store at -25 to -15°C. The product is stable for 2 years. It is recommended to aliquot the reagent upon first thaw to avoid repeated freeze-thaw cycles.
Shipping	Shipped on dry ice.
Labeling Efficiency	For research use only.

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