Luminescent Cell Viability Detection Kit

1.Product Introduction

Chaselection Luminescent Cell Viability Detection Kit（Luminescent Cell Viability，LCV）It is a cell viability analysis method developed using the luciferase chemiluminescence system. Determine the number of living cells with metabolic activity by quantitatively measuring the ATP content after cell lysis. The basic principle of this method is shown in Figure 1. D-Luciferin and stable mutant luciferase in the reagent kit react with ATP in the cell after cell lysis. Live cells can be quantified within a certain number of cells.

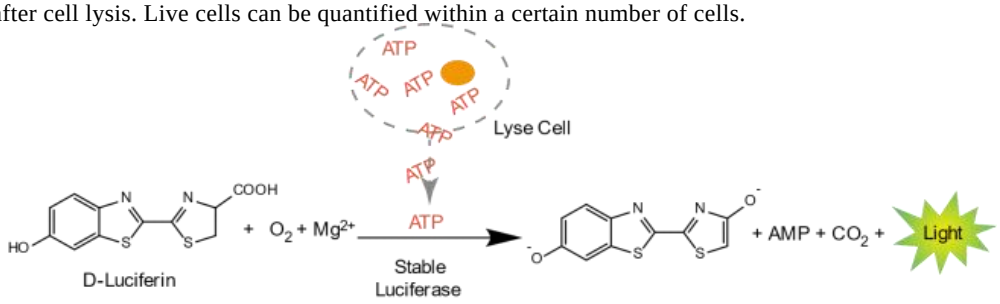


Figure 1. Chaselection Luminescent Cell Viability
Schematic diagram of cell viability detection principle

Chaselection Luminescent Cell Viability detection kit is an open cap and ready to use reagent, which only requires adding an equal volume of reagent to the test well containing cells. Shake at room temperature for 2-5 minutes to fully lyse and mix the cells evenly. After 10 minutes of reaction at room temperature, the luminescence signal reaches its maximum reading value and can be detected for reading. This product is a "glow type" reagent with a half-life of 3-5 hours and excellent sensitivity, suitable for high-throughput cell proliferation and cytotoxicity detection.

2.Product components

Cat No.	Product Name	Size
CY074F0010KIT	Luminescent Cell Viability Detection Kit (100 tests)	10 mL

The substrate of this reagent kit is sufficient for 100 detections in a 96 well plate at a rate of 100 μL per test. Recommend using 100 μL/well cells react with an equal volume of the reagent, with a total reaction volume of 200 μL.

- The Kit Contains：1 × 10 mL Luminescent Cell Viability Detection Reagent

Cat No.	Product Name	Size
CY074F0100KIT	Luminescent Cell Viability Detection Kit (1000 tests)	2*50 mL

The substrate of this reagent kit is sufficient for 1000 detections at a rate of 100μLper test in a 96 well plate. Recommend using 100 μL/well cells react with an equal volume of the reagent, with a total reaction volume of 200 μL.

- The Kit Contains：2 × 50 mL Luminescent Cell Viability Detection Reagent

Cat No.	Product Name	Size
CY074F1000KIT	Luminescent Cell Viability Detection Kit (10000 tests)	20*50 mL

The substrate of this reagent kit is sufficient for 10000 detections at a rate of 100μLper test in a 96 well plate. Recommend using 100 μL/well cells react with an equal volume of the reagent, with a total reaction volume of 200 μL.

- The Kit Contains：20 × 50 mL Luminescent Cell Viability Detection Reagent

3.Storage conditions

- ✧ Below -20 ℃, it can be stored for a long time. It is recommended to use this reagent within its validity period.
- ✧ This reagent kit can withstand repeated freeze-thaw cycles for 10 times (≥ 90% activity).
- ✧ It is recommended to pack and store at -20 ℃ in a dark place for the first time of use.

4. Self provided materials

Single channel pipette or multi-channel pipette; White/black flat bottom luminous detection board; Porous plate vibration plate device; An enzyme-linked immunosorbent assay (ELISA) reader with a luminescent detection module.

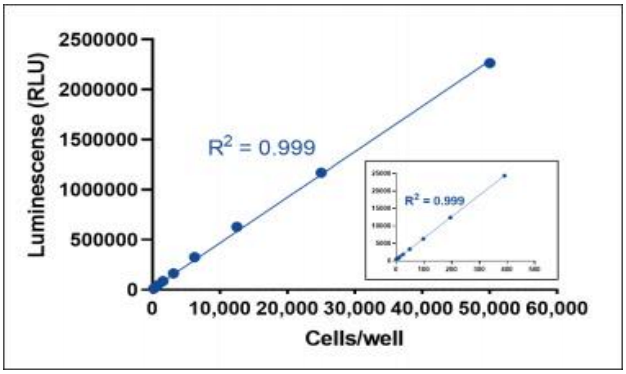
5.Experimental process

Process	Experiment	Operation steps
First	Reagent Preparation	➢ Melt this reagent at 4 ℃ or room temperature in advance.（Do not melt above 25 ℃ to avoid reduced activity） ➢ After the reagent is equilibrated to room temperature, gently invert several times to mix the solution evenly.
Second	Detection	➢ Take out the culture plate of the cells to be tested and let it stand at room tempe rature for about 30 minutes to allow the temperature of the culture plate to balance to room temperature. ➢ Add LCV reagents of the same volume as the test sample.（Recommend adding 100 μL of reagent to 100 μL of the cell culture to be tested） ➢ 10 minShake and lyse the cells for 2-5 minutes to fully lyse and mix well. Place the reaction at room temperature for 10 minutes to maximize the luminescence signal. ➢ Reading luminescent signals on an enzyme-linked immunosorbent assay (ELISA) reader

6.Note

- **ATP pollution:** When there is pollution in the environment, it is easy to introduce exogenous ATP, which can increase the background signal. It is recommended to pay attention to the cleanliness of the experimental table, wear masks and gloves, and ensure that the bottles and caps are not contaminated when packaging and using reagents.
- **Mixing evenly:** This reagent needs to be mixed evenly with the test cells after equal volume addition, so that the cells can fully lyse and release ATP, and obtain stable and high intensity light signals. Due to the influence of the pore size and depth of the microporous plate on the mixing efficiency, different cell characteristics are different. Therefore, it is necessary to optimize the shaking plate scheme, for example, by extending the shaking time or increasing the shaking frequency to improve the mixing and lysis efficiency.
- **Temperature:** The reaction rate of luciferase affects the luminescence intensity and decay rate of the test, and temperature plays a crucial role in enzyme activity. Therefore, before adding the reagent, it is necessary to fully balance the reagent and test sample at room temperature to ensure the consistency of the test results. To avoid the edge effect of the microplate.
- **Chemical factors:** There are differences in the composition of different media, so there will be differences in luminescence intensity and attenuation rate when using different types of media and serum. In addition, the solvents introduced during the treatment of cells with compounds also have an impact on the signal. By using culture media containing solvents as control wells for testing, it was found that the final concentrations of commonly used solvents DMSO, methanol, and ethanol were less than 2% and had no significant effect on the signal.
- **Difference in board type:** Different test boards will also measure different luminous intensities. Using a black test board can reduce the perforation effect, but the loss of light signal is significant; Using a white test board can effectively reduce optical signal loss, but there may be a perforation effect; A white test plate with only a transparent bottom is convenient for observing cell growth, but it is more likely to cause perforation effects between pores.
- **Background effect:** Different culture media and cell types will have certain background effects, and a control group needs to be set up for judgment.

7.Experimental data



The luminescence signal measured using the Chaselection cell viability detection kit is directly proportional to the number of cultured cells, with a linear range spanning three orders of magnitude. Using DMEM medium containing 10% FBS, HEK293 cells were diluted twice starting from 50000 cells in 96 plates and detected as described in Part 5. After adding the reagent for 10 minutes, the Luminescence program of Envision (program details such as Mirror: Luminescence; Em filter: Luminescence 700; Measurement height: 6.5mm; Measurement time: 1 s) was used to detect the luminescence signal. Prepare 2 wells per cell count. The data shows that the luminescence signal obtained from 25 HEK293 cells is three times greater than the background signal in serum containing medium (excluding cells). There is a linear relationship between the luminescence signal and the number of cells per well (0 to 50000) (R2=0.999).

Tips： The above data is for example only, and the actual results may vary depending on the performance of the enzyme-linked immunosorbent assay (ELISA) reader used.