TransDetect® Luminescent Cell Viability Detection Kit

Please read the datasheet carefully prior to use.

Cat. No. FC401

Version No. Version 1.0

Storage: at -20°C away from light for one year.

Description

Adenosine triphosphate (ATP) is an important energy molecule in cells, which can be used to evaluate the metabolic activity of cells and has a good linear correlation with the number of viable cells. Thus, the number of viable cells can be reflected by ATP amount. The kit is designed for detecting cell viability or quantifying the number of viable cells by detecting amount of intracellular ATP via chemiluminescence using the ATP-dependent luciferase-catalyzed luciferin reaction (Figure 1).

The product is ready-to-use reagent with single component. Add an equal volume of reagent to cell culture directly and mix. Perform detection after 10 minutes. The product has high sensitivity, a wide linear range with good linear correlation in the range of 5 to 100,000 cells, and generates a stable "glow-type" luminescent signal which drops no more than 15% within 1 hour of the start of the reaction and has a half-life of up to 3 hours. It is compatible with detection of small amount of samples and high throughput screening detection of large amount of samples.

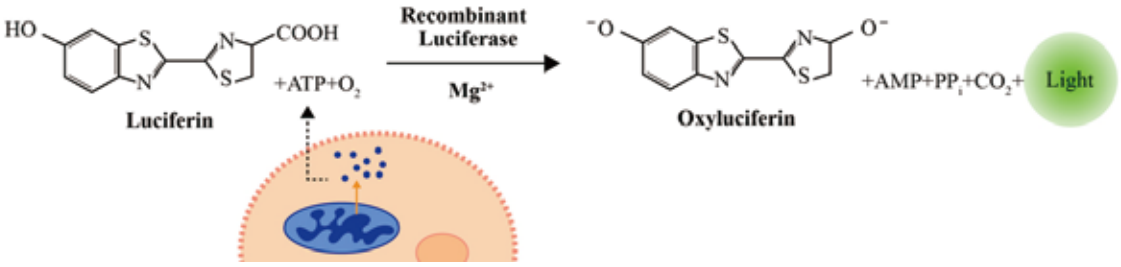

Figure 1. Overview of Cell Viability Detection (Luminescence) Principle

Kit Contents

Component	FC401-01	FC401-02
Cell Viability Reaction Buffer	10 ml	100 ml

Protocols

1.Reagent Preparation

(1) Thaw reagent: at 2~8°C or room temperature.

Note: the thawed reagent can be stored at 2~8°C for 60 days or room temperature for 7 days, maintaining more than 85% activity.

(2) If the unused reagent is not used for a long time, it is recommended to store it at -20°C after aliquoting.

(3) Equilibrate to room temperature, and mix well by turning upside down prior to use.

2.Protocol for the Cell Viability Assay

(1) Take the cells to be tested out of the incubator and incubate at room temperature for at least 15 minutes to allow the culture plate and its contents to completely equilibrate to room temperature.

(2) Add Cell Viability Reaction Buffer, equilibrated to room temperature, equal to volume of cell culture to be tested. If the cells to be tested are cultured in a 96-well plate with a culture volume of 100 µl, add 100 µl of Cell Viability Reaction Buffer per well.

Note: The 96-well plate assay system recommends no more than 100,000 cells per well. Depending on the type of cell, the test effect will vary. For some cells with particularly high ATP amount, chemiluminescence values may not be linearly

related to cell count after the number of cells reaches 100,000, but the reading will still rise.

(3) Mix contents for 3 minutes on an orbital shaker to induce cell lysis at room temperature. Stay still for 5-10 minutes at room temperature to stabilize the luminescent signal.

(4) Record luminescence using a multimode microplate reading with chemiluminescence detection function. Please set the appropriate parameters depend on the manufacturer.

Notes

1.Multiwell plate: it is recommended to use a white or black non-transparent well plate for detection. Different types of well plates have different effects on the test results. The influence between the detection wells of the black plate is small, but the light intensity absorption of the luminescence signal is higher; the influence between the detection wells of the white plate is certain, but the light intensity of the luminescence signal is almost no loss; the transparent plate is conducive to the observation of the cell state during the cell culture process, but the luminescence signal interference between the detection wells is very large. The appropriate plate can be selected for cell culture and detection depend on the different needs of the experiment.

2.Temperature: temperature has a great influence on the luciferase-luciferin reaction rate. Therefore, it is necessary to completely equilibrate the cell culture and the reagents to the same room temperature before testing to ensure the consistency of the test results. For high-throughput detection requirements, the multi-well plate culture system needs to prolong the temperature equilibration time during operation. The stacked culture well plates also need a longer time to equilibrate. The consistency of detection between wells that are not fully equilibrated will be affected, leading to unreliable results.

3.When the solvent content of the drug to be tested is high, it may interfere with the luciferase reaction, thereby affecting the chemiluminescent signal. Solvent interference can be ruled out by setting a cell culture medium control well containing solvent.

4.Environmental contamination: microbes or ATP contamination in the environment will introduce exogenous ATP, which will cause an increase in the background signal. It is recommended to wear a mask and latex gloves during operation, and pay attention to the cleanliness of the experimental table, and carefully open the cover to avoid contamination of the detection system by exogenous ATP.

Figure: Linear correlation between the number of viable cells and luminescence

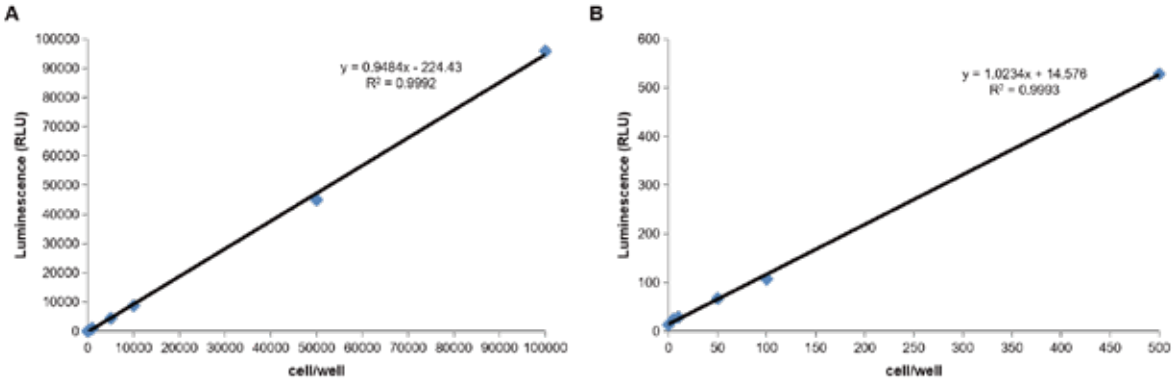

Figure 2. Linear correlation between the number of viable cells and luminescence

Serially dilute Jurkat cells with RPMI 1640 medium containing 10% FBS to prepare cell suspensions of different densities. Add 100 µl of cell suspension to 96-well plate, add an equal volume of Cell Viability Reaction Buffer, and stay still for 10 minutes to detect luminescence. Figure 2A: The number of cells from 0 to 100,000 has a good linear correlation with the luminescence intensity. Figure 2B: The number of cells from 0 to 500 has a good linear correlation with the luminescence intensity.

For research use only, not for clinical diagnosis.

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