CellCounting-Lite® 2.0 Luminescent Cell Viability Assay

DD1101

Version 20.1

Overview to Product

CellCounting-Lite 2.0 is a cell viability detection reagent based on the luciferase system. The reagent contains luciferin with high purity and thermostable luciferase. This product shall be added to the cell culture to make the cell lyse and release ATP, so that the reaction can occur as shown in Figure 1 and a stable "glow" signal shall be sent out. The luminous intensity is directly proportional to the amount of ATP, that is, the number of living cells, within a certain range. Therefore, this product can be used for quantitative detection of the number of living cells.

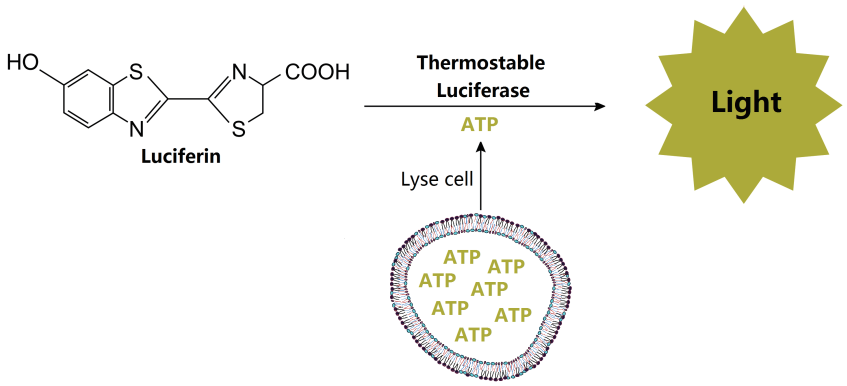

Figure 1. Schematic diagram of CellCounting-Lite 2.0 Detection Principle

As shown in Figure 2, this product is a ready-to-use solution. The same volume of reagent is directly added to the cell culture. The detection can be carried out after 10 min. The "glow type" luminescence produced by this product is very stable, with a half-life period of 4 h. therefore, it is very suitable for high-throughput cell proliferation and cytotoxicity detection. In addition, this product contains special stable ingredients, so that it can be stably preserved at room temperature for 7 days and at 2 - 8˚C for 60 days, avoiding sub packaging or repeated freezing and melting, and improving the convenience of operation.

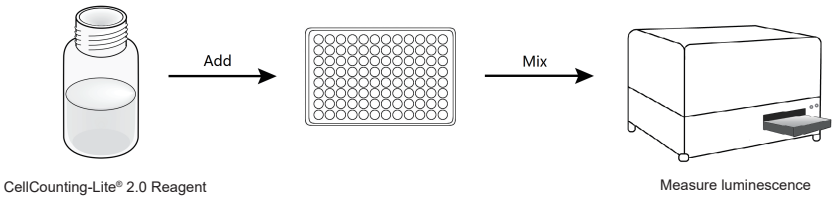

Figure 2. Operation Flow Chart of CellCounting-Lite 2.0

Package Information

Article No.	DD1101-01	DD1101-02	DD1101-03
CellCounting-Lite 2.0 Luminescent Cell Viability Assay	10 ml	100 ml	400 ml

Storage condition

Long-term storage: at -30 to -15˚C. It can be shipped at - 20 to 0˚C.

The melted CellCounting-Lite 2.0 can be preserved at room temperature for 7 days or at 2 to 8˚C for 60 days (>85% activity).

After 5 cycles of freezing and melting, it can still keep stable. For the reagent that is not used for a long time, to store it at - 20˚C is suggested.

Self-provided material

Single-channel pipette or multi-channel pipette; White / Black flat bottomed luminous detection plate; Perforated plate vibrating device; Microplate reader with a luminescence detection module.

Operation process

Reagent preparation

1. **Melt:** The product shall be placed at 2 to 8℃ or room temperature for melting. The product can also be placed in a 22℃ water bath for melting, but the water temperature shall not exceed 25℃, which shall be noted.
2. **Equilibrium to room temperature:** If the product is melted not at room temperature, it can placed in a 22℃ water bath for a period of time before use, so as to be balanced to room temperature. Generally, it takes about 10 min for 10 ml package; It takes about 30 min for 100 ml package; It takes about 100 min for 400 ml package;
3. **Gently** invert it 5 times before use, to mix the solution evenly.

Detection steps

1. Take out the cell culture plate to be tested from the incubator and place it at room temperature for 30 min to keep the temperature of the plate balanced to room temperature.
2. Add CellCounting-Lite 2.0 which is equal to the volume of the cell culture to be **tested and balanced to room temperature.** For example, when using a 96-well culture plate, add 100 μl CellCounting-Lite 2.0 into 100 μl cell culture to be tested.
3. Shake and mix it for 2 - 5 min, so as to make the cells fully lysed; It shall be placed at room temperature for 10 min to stabilize the luminescence signal. Then, the detection can be carried out.

Precautions

1. **Temperature:** The intensity of luminescence and rate of decay depend on the reaction rate of luciferase. Temperature has a direct effect on the enzyme reaction rate, so this product and cell culture shall be balanced to room temperature before adding samples, to ensure the consistency of test results. Pay attention to batch operations, stacked perforated plate require more time to balance to room temperature than monolayer placed perforated plates, uneven temperature of perforated plate may occur due to inadequate balance to cause a gradient effect between the center and edge of the perforated plate.
2. **Chemical factors:** The chemical compositions of different culture mediums shall be different. Therefore, the intensity and attenuation rate of luminescence shall be slightly different when different types of culture mediums and serums are used. In addition, the solvents introduced in the treatment of cells by the compounds may also affect the luminescence. The interference of solvent can be eliminated by setting the control well of culture medium containing solvent. When the final concentration of common solvents such as DMSO, methanol and ethanol is < 2% through test, there is no significant effect on the luminescence signal.
3. **Perforated plate:** We recommend using flat bottomed non-transparent light-emitting detection plate, and the luminescent intensity measured by different types of perforated plates shall be different. The use of black perforated plate can effectively reduce the influence between wells, but the optical loss is large; The use of white perforated plate can effectively reduce the optical loss, but there shall be a certain interference between wells; The non-transparent cell culture plate with transparent bottom is conducive to direct observation of cell growth, and can also be used for luminescence detection, but this type of perforated plate shall increase the interaction between wells. It is suggested to select it from different types of perforated plates according to the experimental needs.
4. **Mix well:** Sufficient reaction can be made only when this product is completely mixed with the cells to be tested and the cells can be fully lysed. Compared with adherent cells, suspension cells are easier to mix evenly, which is conducive to cell lysis and ATP release. If the sample to be tested is a suspension cell, the mixing step can be omitted after adding the sample, which has no significant effect on the detection results; If the sample is adherent cell, the mixing and splitting efficiency can be improved by prolonging the plate vibrating time or increasing the plate vibrating frequency. As the well size and liquid depth of the perforated plate shall affect the mixing efficiency, it is more difficult for 384-well plate to mix than 96-well plate, so it is necessary to pay attention to the adjustment of plate vibrating parameters. It is suggested to determine the degree of cell lysis by microscopic observation, in order to optimize the plate vibrating scheme.
5. **Microbial pollution:** Microbial pollution in the environment shall lead to the introduction of exogenous ATP, resulting in the increase of background signal. We suggest wearing masks and latex gloves during operation, and attention shall be paid to the cleanliness of the test table, and the cover shall be carefully opened.

Appendix I: The linear relationship between the number of cells and luminescence

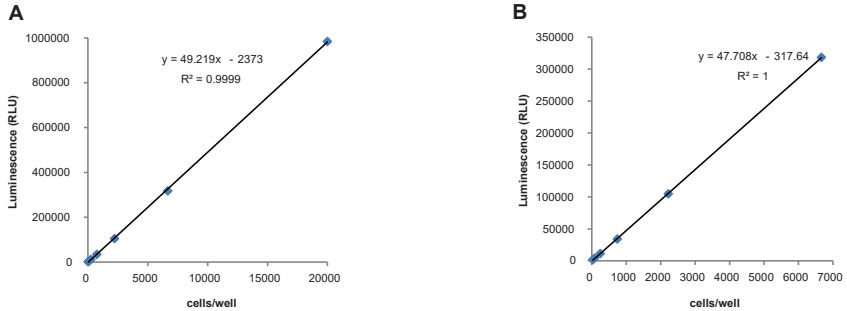

Figure 3. The linear relationship between the number of cells and luminescence

Figure 3. The linear relationship between the number of cells and luminescence Jurkat cells shall be diluted with RPMI1640 culture medium containing 10% FBS for 3 times to prepare cell suspension with different densities. 100 μ L cell suspension shall be added into 96-well plate (Figure 3A), or 25μl cell suspension shall be added into 384-well plate (Figure 3B). After adding equal volume of CellCounting-Lite 2.0, the luminescence shall be detected after standing for 10 min.