CellCounting-Lite 3D Luminescent Cell Viability Assay

NB-54-0393-01
NB-54-0393-02
NB-54-0393-03

CellCounting-Lite 3D Luminescent Cell Viability Assay

#Cat: NB-54-0393-01 Size: 10ml
#Cat: NB-54-0393-02 Size: 100ml
#Cat: NB-54-0393-02 Size: 400ml

Overview to Product

CellCounting-Lite 3D is a cell viability detection reagent based on the luciferase system. This reagent contains luciferin with high purity, thermostable luciferase and optimized reaction reagent, which makes the lysis ability stronger, and it is more suitable for micro tissue cell mass samples under 3D cell culture conditions. This product shall be added to the cell culture to make the cell mass lyse and release ATP, so that the reaction can occur as shown in Figure 1 and a stable optical 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.

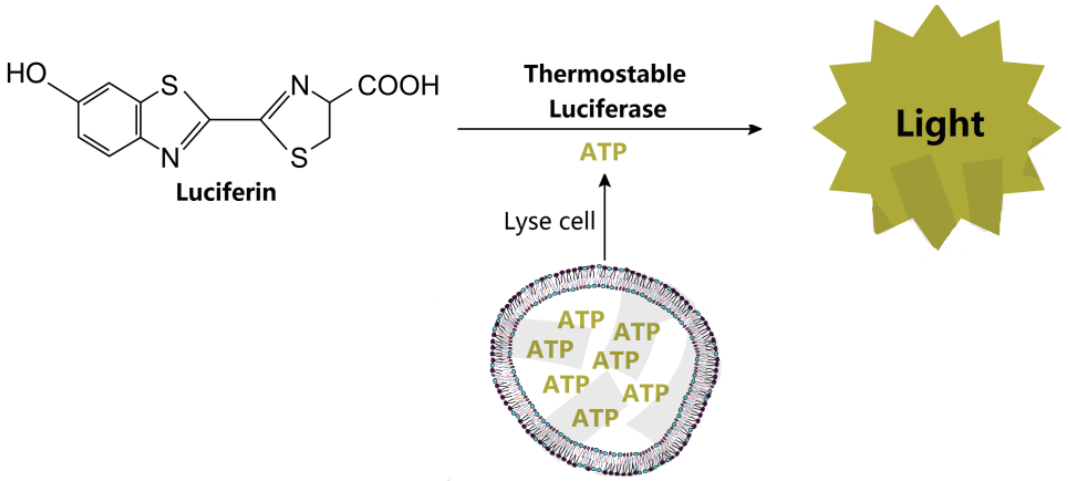

Figure 1. Schematic diagram of CellCounting-Lite 3D Detection Principle

As shown in Figure 2, this product is a ready-to-use solution. Add the equal volume of reagent directly into the cell culture, shake and mix them for 5 min until the cell mass fully lysed. The detection can be carried out after incubating for 25 min. The "glow type" signal produced by this kit is very stable, with a half-life period of 3 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 to 8℃ for 60 days, avoiding sub packaging or repeated freezing and melting, and improving the convenience of operation.

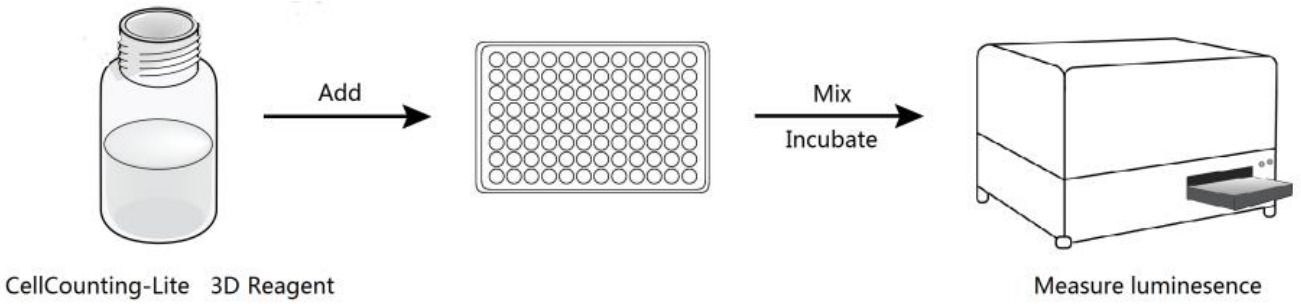

Figure 2 Operation Flow Chart of CellCounting-Lite 3D

Package Information

Article No.	NB-54-0393-01	NB-54-0393-02	NB-54-0393-03
CellCounting-Lite 3D Luminescent Cell Viability Assay	10ml	100ml	400ml

Storage condition

Long-stem storage: -30 ~ -15℃; Transport conditions: ≤0℃. The melted CellCounting-Lite 3D can be preserved at room temperature for 7 days or at 2 - 8℃ for 60 days (>85% activity). After 10 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℃ is suggested.

Experiment preparation

Self-provided material

Single/multi-channel pipette; It is applicable to white / black perforated plate for 3D cell culture; 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. Reagent preparation .
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. Detection steps .
2. Add CellCounting-Lite 3D 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 3D into 100 µl cell culture to be tested.
3. The cell mass shall be fully lysed by violently shaking for 5 min; It shall be placed at room temperature for 25 min to stabilize the luminescence signal. Then, the detection can be carried out.

Precautions

1. **Temperature**: The intensity of luminescence and rate of decay depends 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 special 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**: 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 cell mass 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. **ATP content in the cell:** ATP content in the cell: The ATP contents of different types of cells are different. In addition, the ATP mental needs. concentration of the micro tissue cell mass decreases gradually from the outer living cell layer to the central non-living cell layer, and this relative change also varies with the types of cells. Under the condition of 3D cell culture, it is easy to have excess single-well sample (i.e. ATP concentration exceeds the upper limit of detection by 10 μm). In case of occurrence of this situation, we recommend using smaller micro tissue cell mass as much as possible or diluting the sample before detection. It is necessary to carry out the detection immediately after dilution to avoid ATP degradation caused by too long operation time, to which special attention shall be paid.
4. **Cell culture volume**: Ensure that the total volume of the cell culture to be tested and the tested compound is less than half of the well volume, and there shall be no cross contamination between the wells after mixing with the equal volume of this product.
5. **Mix well**: the reaction can be fully performed only when the product is completely mixed with the cell mass to be tested and the cells are fully lysed, so as to obtain the best detection performance. If the cell mass is not fully lysed, resulting in uneven luminescent value between the complex wells, it can be optimized by increasing the vibrating amplitude of the plate or prolonging the incubation conditions. In addition, 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 evenly than the 96-well plate. We suggest selecting the plate vibrating instrument with horizontal oscillation mode. The specific vibration plate parameters and incubation time can be adjusted according to the actual cell type and 3D cell culture conditions.
6. **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.