Luminescent 3D Cell Viability Assay Reagent

Introduction

ATP is an important source of energy in cells and can be used to measure cellular metabolic activity. In addition, ATP levels are positively correlated with the number of viable cells. Therefore, ATP content can be used to reflect the number of living cells.

Compared with traditional 2D cell culture, 3D cell culture can simulate the biological environment in vivo better. This technology is widely used in biomedical research, drug screening, tissue engineering, and regenerative medicine. The Luminescent 3D Cell Viability Assay Reagent is a product that uses firefly luciferase to detect 3D cell viability. This reagent contains a thermostable firefly luciferase and highly purified luciferin substrate . When added to 3D cells, the reagent lyses the cells, releasing ATP, which then reacts with the substrate under luciferase catalysis to produce a stable luminescent signal.

This product is optimized for strong lysis capacity, stronger than reagents such as K2040 (designed for standard 2D cell ATP detection), so it can effectively penetrate into the center of 3D cell spheroids. Compared with other 3D cell viability assay reagents, this product provides a simple, quick and highly sensitive method.

Components and Storage

Components \ Size	100 Tests	500 Tests	1000 Tests	2500 Tests	Storage
Luminescent 3D Cell Viability Assay Reagent	10 mL	50 mL	100 mL	250 ml	-20°C/-80°C away from light

Shipping: Dry ice **Shelf life: 1 year in -20°C. More than 1 year in -80°C.**

Protocol

1. **Cell seeding:** It is recommended to use plate suitable for luminescence detection for cell seeding, such as white or black cell culture plates (better white). Seeding 100 µL of cells per well using a 96-well plate is suitable for luminescence detection (e.g., 25 µL of cells per well using a 384-well plate). The number of cells can be optimized depending on the specific experiment. A blank control well (containing only the culture medium and other necessary things with no cells) is also recommended to be set up. Culture cells according to the 3D cell culture method.

***Note:** If using clear plates, after lysis and incubation, transfer the contents to white or black plates just before the detection, better

white.

2. **Drug treatment (Optional):** If needed, treat cells with the drug of interest according to the experimental design. At the same time, set up a negative control group (including cells, culture medium and drug solvents).

3. **Assay Reagent Preparation:** Thaw the Luminescent 3D Cell Viability Assay Reagent and equilibrate it to room temperature in advance. If needed, thaw it in a 22°C water bath. Gently invert several times to mix the reagents.

***Note:**

a) Luciferase activity is temperature sensitive. Therefore, this reagent needs to be equilibrated to room temperature before use.

b) Be careful when opening the sealing cap of the bottle if using water bath, dry off the water on the surface of the bottle to avoid ATP contamination.

c) After the first thaw, this product can be dispensed into aliquots to avoid repeated freeze-thaw cycles. Pay attention to using containers that do not contain ATP contamination.

4. **Detection:**

1) Remove the plate from the cell culture incubator. Equilibrate it at room temperature for 30 min.

2) Add Luminescent 3D Cell Viability Assay Reagent equal to the volume of culture medium in each well. For a 96-well plate, add 100 µL Luminescent 3D Cell Viability Assay Reagent to 100 µL culture medium in each well. (Add 25 µL reagent to 25 µL culture medium for 384 plate.)

3) Shake at room temperature for 5 min for cell lysis.

4) Incubate at room temperature for 25 min to stabilize the luminescence signal.

5) Record the luminescence signal. The detection instrument settings vary depending on the manufacturer. As a guideline, use a detection time of 0.25–1 s per well.

***Note:** The time for reagent penetration into 3D cells and signal stabilization depends on cell type, culture duration, and 3D spheroid size. If necessary, detection can begin after adding the reagent → shaking for 5 min → incubating for 5 min. Then record luminescence every 5 min. Use the stable signal value as experimental data finally.

5. **ATP Standard Curve (Optional):**

ATP standard curve is not required for routine cell viability assays. If needed, it is recommended to use the same plate for samples.

1) Dilute the ATP solution with cell culture medium to produce a range of ATP concentrations. 0, 10, 50, 100, 500, 1000, 2000, 3000 and 4000 nM series concentrations can be set for the initial assay.

***Note:** This kit does not provide the ATP standard solution.

2) Add the various concentrations of ATP standards in a 96-well plate in 100 µL. Then add Luminescent 3D Cell Viability Assay Reagent equal to the volume of ATP standard in each well.

***Note:** ATP standards should be tested immediately after dilution, as enzymes in serum (e.g., ATPase) can deplete ATP.

3) Shake at room temperature for 5 min to mix the solution.

4) Incubate at room temperature for 25 min to stabilize the luminescence signal.

5) Record the luminescence signal. The detection instrument settings vary depending on the manufacturer. As a guideline, use a detection time of 0.25–1 s per well.

Note

1. This reagent has a shelf life of one year when stored at -20°C. When stored at -80°C in a dark environment, it can be preserved for an even longer period.
2. The reagent contains luciferase, so repeated freeze-thaw cycles need to be avoided as much as possible. After testing, three freeze-thaw cycles have no significant effect on the activity. After being subjected to repeated freeze-thaw cycles for 7 times, its efficacy decreased by approximately 5% to 20% (for different condition, the decrease is different). If you have purchased a large package and need to aliquot it, the containers must not be contaminated with ATP.
3. High levels of drug solvents in the system may affect the enzymatic reaction. It is recommended to set up negative controls (containing cells, media, and drug solvents) to exclude solvent interference. In general, 2% DMSO in the system does not affect the test results.
4. It is recommended to use white or black cell culture plates to seed cells to avoid interference between adjacent wells.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com