TECHNICAL MANUAL

CellQuant-Lux 3D Luciferase Assay System

- **SKU CODES:** ASRV00012-10 / ASRV00012-100 /ASRV00012-400
- **SIZE:** 10ml / 100ml / 400ml
- **DETECTION PRINCIPLE:** Luminescence
- **RUO:** Research-Use-Only

Revised: 11/2025

CellQuant-Lux 3D Luciferase Assay System

Please read entire manual carefully before starting experiment.

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1. Product Description

CellQuant-Lux 3D is a single-reagent, homogeneous assay specifically formulated to accurately determine the number of viable cells in complex 3D cell culture models (microtissues and spheroids). It quantifies the ATP present, which serves as a marker for metabolically active cells. This ready-to-use reagent is based on the highly sensitive luciferase system and is formulated with an optimized reaction composition and significantly stronger lytic capacity to effectively penetrate and lyse dense microtissue cell masses.

The assay is compatible with multiwell-plate formats and the homogeneous "add-mix-measure" procedure makes it ideal for automated High-Throughput Screening (HTS) using cell proliferation and cytotoxicity assays. Cell washing, removal of medium, and multiple pipetting steps are not required.

Detection Principle: The foundation of the assay is the luciferase reaction, shown below:

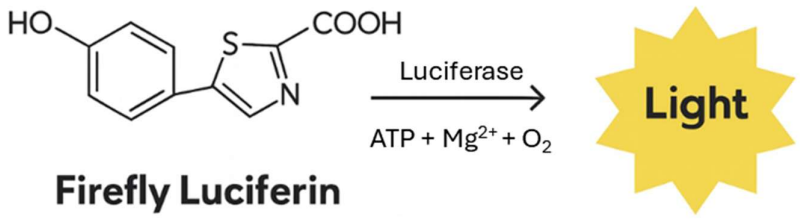

Figure 1. Schematic of CellQuant-Lux 3D Detection Principle. The mono-oxygenation of luciferin is catalyzed by the thermostable luciferase in the presence of magnesium ions (Mg^{2+}), molecular oxygen (O_2), and ATP. The ATP required for this reaction is contributed directly by the viable cells following reagent-induced lysis.

Simple, Fast Workflow & HTS Ready

The single-reagent addition and "add-mix-measure" format results in complete cell mass lysis and the generation of a luminescent signal proportional to the amount of ATP released. The amount of ATP is directly proportional to the number of viable cells present in culture. The reagent contains high-purity luciferin and a thermostable luciferase that generates a stable "glow-type" luminescent signal and ensures improved performance across varied 3D assay conditions.

3D Cell Culture Performance

CellQuant-Lux 3D is optimized for 3D culture conditions. Its enhanced lytic capacity ensures accurate determination of viability in dense microtissues where lysis by standard 2D reagents may be incomplete.

While the relationship between the seeded cell number and luminescent output in 3D culture may become curvilinear over time (due to nutrient diffusion limitations or necrosis in the center of large spheroids), the assay effectively reports the number of viable cells by accurately quantifying the released ATP.

Protocol and Stability

The assay procedure is simple and fast: Add the equal volume of reagent directly into the cell culture, shake and mix them for 5 minutes until the cell mass is fully lysed. The detection can be carried out after incubating for 25 minutes. Stable Signal: The "glow type" signal produced is highly stable, with an extended half-life period of 3 hours. This extended half-life provides flexibility for continuous or batch-mode processing of multiple plates and eliminates the need for automated reagent injectors.

Unmatched Reagent Stability

This product contains special stable ingredients, allowing it to be preserved stably at room temperature for 7 days and at 2℃ to 8℃ for up to 60 days. This exceptional stability avoids the inconvenience of sub-packaging or repeated freezing and thawing, improving operational convenience.

A Great High-Performance Alternative

CellQuant-Lux 3D Luciferase Assay System is a great alternative to Promega CellTiter-Glo 3D® Assays.

2. Kit Contents & Storage

Product	Code	Pack Size	Assays Per Pack
CellQuant-Lux 3D	ASRV00012-10	10ml	100 assays/96-well plate 400 assays /384-well plate
CellQuant-Lux 2D	ASRV00012-100	100ml	1000 assays/96-well plate 4000 assays /384-well plate
CellQuant-Lux 2D	ASRV00012-400	400ml	4000 assays/96-well plate 16,000 assays /384-well plate

Transport conditions: ≤0℃. Long-term storage: at -30 to -15℃. Once thawed, CellQuant-Lux 3D can be kept at room temperature for 7 days or at 2 to 8℃ for 60 days (>85% activity). It can also maintain activity for up to 10 freeze/thaw cycles. For long-term storage, keep at - 20℃.

Additional Equipment Required:

- Single/multi-channel pipettor
- Opaque-walled multiwell plates suitable for 3D cell culture (e.g., white or black plates)
- Microplate reader with a luminescence detection module
- Orbital plate shaker
- 22°C water bath

3. Protocol

Reagent Preparation

1. **Thawing**: Incubate the CellQuant-Lux 3D at 2 to 8℃ or room temperature to thaw. The product can also be incubated at 22℃ in a water bath but the temperature should not exceed 25℃.
2. **Equilibrate** to room temperature: If the product is not thawed at room temperature, it should be placed in a 22℃ water bath to equilibrate to room temperature. Generally, it takes about 10 min for 10 ml pack size, 30 min for 100 ml pack size or 100 min for 400 ml pack size.
3. **Mix**: Gently invert 5 times before use, to mix the solution evenly.

Detection steps

1. Prepare multiwell plates containing your microtissues in culture medium (we recommend the use of opaque wall microplates compatible with your luminometer). Optimize microtissue size, number as well as cell culture conditions prior to experimentation.
2. Add the test compound(s) to your experimental wells and incubate according to your established cell culture protocol.
3. Optional Background Control: Prepare control wells containing only culture medium (without cells) to determine the background luminescence signal.
4. Remove the cell culture plate to be tested from the incubator and equilibrate at room temperature for 30 min.
5. Add equal amount of CellQuant-Lux 3D which is equal to the volume of the cell culture to be tested. For example, when using a 96-well culture plate, add 100 µl CellQuant-Lux 3D to 100 µl cell culture to be tested.
6. Vigorously mix on a plate shaker (orbital) for 5 minutes to lyse the cells.
7. Incubate at room temperature for 10 min to stabilize the luminescence signal.
8. Detect luminescence.

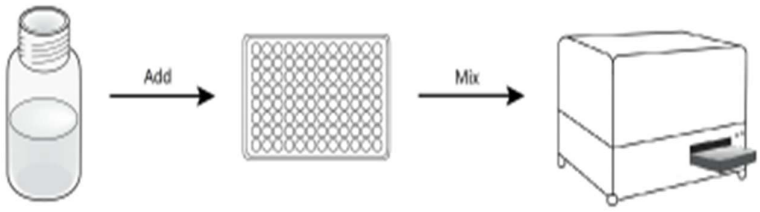

Figure 2. Short Protocol for CellQuant-Lux 3D. The "add-mix-measure" format is achieved through the single-reagent addition of CellQuant-Lux 3D. This simple format enables cell lysis and the generation of a luminescent signal directly proportional to the amount of ATP present, which in turn reflects the number of viable cells in culture. As a ready-to-use solution, simply add the equal volume of reagent directly into the cell culture. Shake and mix vigorously 5 minutes to ensure the cell mass is fully lysed. The detection can then be carried out after incubating for a further 25 minutes. CellQuant-Lux 3D utilizes a thermostable luciferase system and optimized ingredients to generate a highly stable "glow-type" luminescent signal.

Additional Protocol Considerations

1. **Temperature Equilibration:** Temperature control is critical, as both the luminescence intensity and the rate of decay are directly dependent on the reaction rate of the thermostable luciferase. To ensure the consistency and reliability of your assay results, it is mandatory that both the CellQuant-Lux 3D reagent and the cell culture plate are fully equilibrated to room temperature (RT) before adding the reagent. Batch Operation Note: When processing multiple plates or using stacked plates, allow extra time for equilibration compared to single, monolayer plates. Inadequate temperature balance can lead to an uneven temperature distribution across the plate (a "gradient effect" between the center and edge), which introduces variability and inconsistency in test results.
2. **Media, Solvents & Compounds:** The chemical compositions of different culture mediums and serums can vary, leading to slight differences in the resulting luminescence intensity and attenuation rate. Additionally, any solvents introduced in the process of treating cells with test compounds may also affect the luminescence signal. Mitigation: Interference from solvents can be accurately accounted for by setting up control wells containing culture medium and the solvent at the appropriate concentration, but without cells. Recommendation:

Testing has confirmed that when the final concentration of common solvents (such as DMSO, Methanol, and Ethanol) is maintained at 2%, there is typically no significant effect on the luminescence signal.

3. ATP content in the cell: he ATP content varies between different cell types. Furthermore, in 3D microtissue cell masses, the ATP concentration gradually decreases from the outer, viable cell layer to the central, non-viable (necrotic) core; this relative change also varies depending on the specific cell type. Under 3D culture conditions, it is possible to have an excess single-well sample where the ATP concentration exceeds the upper limit of the assay's detection range. If this occurs, we recommend using smaller microtissue cell masses or diluting the sample before detection. If dilution is necessary, the detection must be carried out immediately after dilution to prevent ATP degradation caused by a prolonged operation time, which requires special attention.
4. **Multiwell Plate Selection:** We recommend using standard opaque-walled multiwell plates (e.g., white or black flat-bottom plates) specifically designed for luminescence measurements. It is important to note that the luminescent intensity measured will differ based on the type of plate used: White Plates: Effectively reduce optical loss, leading to a higher signal, but may exhibit a certain degree of interference between wells (cross-talk). Black Plates: Effectively reduce cross-talk between wells, but result in a greater optical loss, leading to diminished signal intensity. Alternative Plates: Opaque-walled cell culture plates with transparent bottoms are also suitable for luminescence detection and allow for microscopic visualization of cell growth. However, assays performed in these plates will typically exhibit diminished signal intensity and increased cross-talk between wells. Plates should be selected based on the specific requirements of the experiment.
5. **Cell culture volume:** Ensure that the total volume of the cell culture medium and any tested compounds in each well is less than half of the total well volume. This prevents cross-well contamination when the equal volume of CellQuant-Lux 3D reagent is subsequently added and mixed.

6. **Mixing Efficiency and Cell Lysis**: The optimal detection performance is achieved only when the CellQuant-Lux 3D reagent is completely mixed with the cell mass and the cells are fully lysed. If the cell mass is not fully lysed, it may result in uneven luminescent values within complex wells. This can be optimized by increasing the plate readers orbital amplitude or prolonging the incubation time. Since well size and liquid depth affect mixing efficiency, making it more difficult to achieve even mixing in 384-well plates compared to 96-well plates, attention must be paid to adjusting shaking parameters. We suggest selecting an orbital plate shaking instrument. The specific vibration parameters and incubation time should be adjusted according to the actual cell type and 3D cell culture conditions.
7. **Microbial ATP Contamination:** Microbial pollution in the environment introduces exogenous ATP, resulting in an undesirable increase in the background luminescence signal. Strict aseptic technique is essential to prevent this contamination of the CellQuant-Lux 3D Reagent. Handling: Wear masks and latex gloves during operation to minimize ATP introduction from personal contact. Workspace: Pay attention to the cleanliness of the test table; we recommend wiping surfaces with appropriate disinfectants (like a 10% bleach solution, if used for cleaning) and ensuring the area is dry before beginning the assay. Reagent Access: Avoid inserting pipettes or pipette tips into the CellQuant-Lux 3D Reagent bottle multiple times. When adding reagent to the plate, the cover shall be carefully opened and handled. Waste Management: Discard any unused reagent that has been dispensed or exposed to the environment; do not return it to the original bottle.

4. Important notes

1. This kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.