TECHNICAL MANUAL

CellQuant-Lux 2.0 Luciferase Assay System

- **SKU CODE:** ASRV00011-10 / ASRV00011-100 /ASRV00011-400
- **SIZE:** 10ml / 100ml / 400ml
- **DETECTION PRINCIPLE:** Luminescence
- **RUO:** Research-Use-Only

Revised: MM/YYYY

CellQuant-Lux 2.0 Luciferase Assay System

Please read entire manual carefully before starting experiment.

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1. Product Description

CellQuant-Lux 2.0 is a highly sensitive, single-reagent assay designed for quantitative detection of viable cells by measuring ATP via luminescence. This cell viability detection reagent utilizes an optimized luciferase system containing high-purity luciferin and thermostable luciferase. Upon addition to the cell culture, the reagent quickly lyses the cells, releasing ATP. This ATP drives the luciferase reaction, resulting in a stable "glow" signal. The luminescence intensity generated is highly linear across a broad detection range (e.g., $R^2 > 0.99$), ensuring accurate quantitative detection of viable cells.

Simple, Fast Workflow & HTS Ready

As a ready-to-use solution, CellQuant-Lux 2.0 features a simple "add-mix-measure" protocol. Detection is fast, requiring a brief 2–5 minute shake/mix followed by a 10-minute incubation to stabilize the luminescence signal.

The resulting "glow type" luminescence is highly stable, with an exceptionally long half-life period of 4 hours. This makes the assay perfectly suited for High-Throughput Screening (HTS) applications in viability, cell proliferation and cytotoxicity studies.

Unmatched Reagent Stability

The reagent contains special stable ingredients that ensure superior operational convenience:

- Working Stability: Remains stable at room temperature for up to 7 days and at 2℃ to 8℃ for up to 60 days (retaining 85% activity).
- Freeze-Thaw Robustness: Demonstrates stability for up to 5 cycles of freeze/thaw, avoiding the inconvenience of sub-packaging and repeated freeze-thaw damage.

A Great High-Performance Alternative

CellQuant-Lux 2.0 Luciferase Assay System is a great alternative to Promega CellTiter-Glo® and CellTiter-Glo 2.0® Assays.

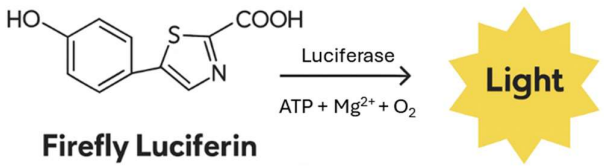

Figure 2. Schematic of CellQuant-Lux 2.0 Detection Principle. The mono-oxygenation of luciferin is catalyzed by the thermostable luciferase in the presence of magnesium ions (Mg^{2+}), molecular oxygen (O_2), and ATP. The ATP required for this reaction is contributed directly by the viable cells following reagent-induced lysis.

2. Contents, Storage and Expiry

	ASRV00011-10	ASRV00011-100	ASRV00011-400
CellQuant-Lux 2.0 Luminescent Cell Viability Assay	10 ml	100 ml	400 ml

Long-term storage: at -30 to -15℃.Transport conditions: ≤0℃ .

Once thawed, CellQuant-Lux 2.0 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 5 freeze/thaw cycles.

For long-term storage, keep at - 20℃.

Additional Equipment Required:

- Single/multi-channel pipettor
- White/black cell culture plate
- Microplate reader with a luminescence detection module
- 22°C water bath

3. Protocol

Reagent Preparation

1. **Thawing**: The product should be placed at 2 to 8℃ or room temperature. The product can also be placed in a 22℃ water bath but the water 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

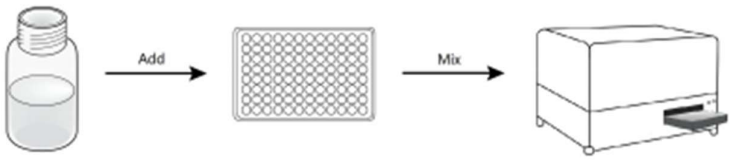

Figure 2. Short Protocol for CellQuant-Lux 2.0. The fundamental mechanism of the assay is based on the luciferase reaction (Figure 1). The "add-mix-measure" format is achieved through the single-reagent addition of CellQuant-Lux 2.0. This simple format immediately results in cell lysis and the generation of a luminescent signal that is directly proportional to the amount of ATP present, which in turn reflects the number of viable cells in culture. CellQuant-Lux 2.0 utilizes a thermostable luciferase system and optimized ingredients that generate a highly stable "glow-type" luminescent signal. The extended half-life of the resulting signal is exceptionally long, lasting 4 hours. This stability is a key feature, eliminating the need for automated reagent injectors and offering superior flexibility for continuous or batch-mode processing of multiple plates in High-Throughput Screening (HTS).

The simplicity of the **single-reagent-addition** format significantly **reduces pipetting errors** and variability that are often introduced during the multiple steps required by traditional ATP-measurement methods.

Detection steps

1. Remove the cell culture plate to be tested from the incubator and equilibrate at room temperature for 30 min.

2. Add equal amount of CellQuant-Lux 2.0 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 2.0 to 100 µl cell culture to be tested.

3. Mix on a plate shaker (orbital) for 2 - 5 minutes to lyse the cells.

4. Incubate at room temperature for 10 min to stabilize the luminescence signal.

5. Detect luminescence.

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 should be equilibrated 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.

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.

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

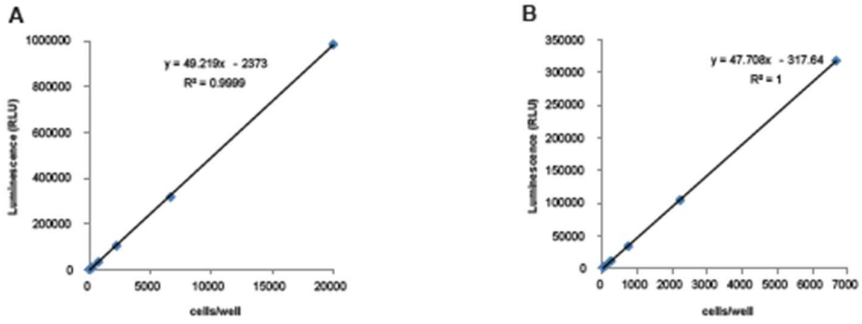

Figure 3. Linear relationship between cell number and luminescence. Jurkat cells were serially diluted in RPMI1640 culture medium supplemented with 10% FBS to generate suspensions of varying densities. Cell suspensions were seeded into either a 96-well plate (100 µL/well; **Figure 3A**) or a 384-well plate (25 µL/well; **Figure 3B**). Following the addition of an equal volume of CellCounting-Lite 2.0, plates were incubated for 10 minutes prior to luminescence detection.

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.