Testing a Novel Real Time Cell Viability Assay: Comparison to ATP Assay and Compatibility for Multiplexing

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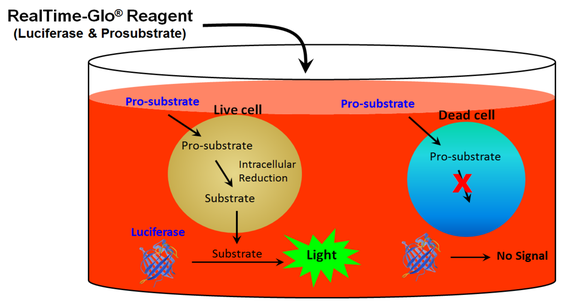
1. Introduction

Recently developed assay technologies make it possible to use multi-well plate readers to measure the number of live or dead cells in culture in real time over a period of days. Live cells are measured in real time by adding a reagent containing a shrimp-derived luciferase and a pro-substrate directly to the culture medium. Only viable cells can convert the pro-substrate into a luciferase substrate and generate light. The real time viability assay is non-toxic to cells, so viable cells remain in the sample well following measurement of the live cell signal. In addition to providing real time kinetic measurements that are valuable for assay development and characterization activities, multiplexing with other assays (e.g. dead cell staining, apoptosis, oxidative stress markers, reporter gene assays or RNA extraction) provides a time saving approach and statistical advantage inherent in taking measurements from the same sample of cells.

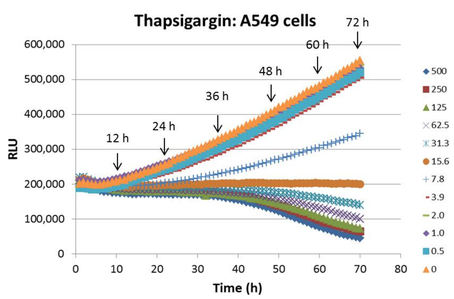
2. Real-Time Assay to Measure Live Cells

Pro-substrate and shrimp-derived luciferase are added as a reagent directly to cell culture medium. Only live cells convert pro-substrate to a luciferase substrate and generate light. Luminescence is proportional to the number of live cells.



3. Three-Day Time Course Measuring Live Cell Number

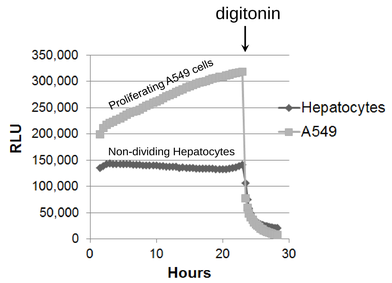
Luminescence intensity is proportional to the number of live cells. A549 cells were treated with various doses of Thapsigargin. RealTime-Glo™ Reagent was added, and luminescence measured every hour for 3 days. Vehicle control cells grow over time. Cells treated with high concentrations of Thapsigargin die.

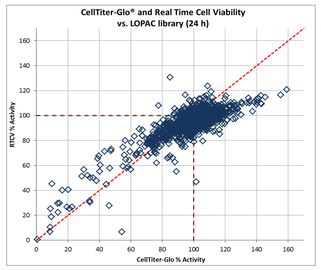


4. Loss of "Real Time" Signal Upon Cell Death & Correlation with ATP Assay Results

Luminescent signal from the RealTime-Glo™ Assay decreases immediately after addition of digitonin to kill cells.

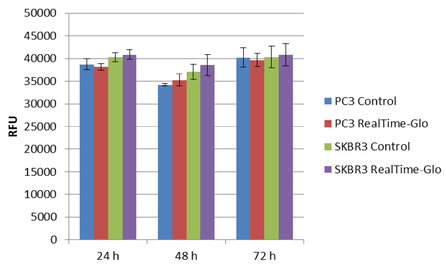
There is good correlation between the RealTime-Glo™ and CellTiter-Glo® ATP Assays from screening LOPAC library.





5. RealTime-Glo™ Reagents are Not Toxic

PC3 or SKBR3 cells were cultured in the presence or absence or RealTime-Glo™ Reagent for 3 days. Samples were tested for membrane integrity (leakage of protease into culture medium) using CytoTox-Fluor™ Cytotoxicity Assay. The presence of RealTime-Glo™ Reagent for 3 days does not affect viability.



6. Multiplexing Orthogonal Viability Assays on Same Samples Demonstrating Similar Toxin EC_{50} Values

Add RealTime-Glo™ Assay Reagent to A549 cells treated with Thapisgargin

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Luminescence can be recorded continuously for 3 days (optional)

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Add CellTiter-Glo® Reagent (lyses cells, eliminates signal from RealTime-Glo™ Assay and detects ATP)

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Incubate 10 min

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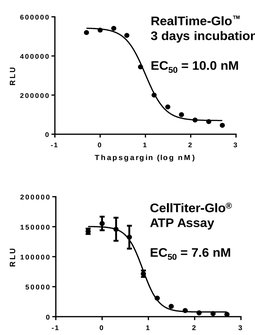
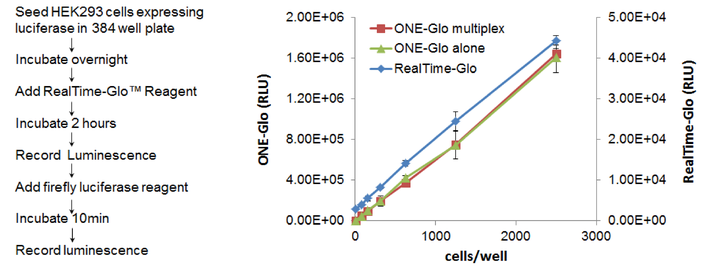
Record luminescence from the same plate to quantify ATP as orthogonal viability marker



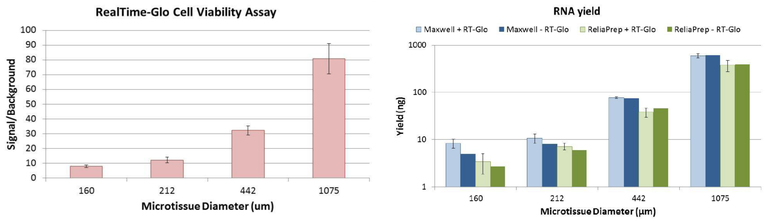
7. Multiplexing Viability and Luciferase Reporter Assays

Firefly luciferase reporter assay signal from 2500 cells is not affected by the presence (1.61 +/- 0.15 x 10^6 RLU; red squares) or absence (1.64 +/- 0.05 x 10^6 RLU; green triangles) of RealTime-Glo™ Reagent.



8. Multiplexing RNA Extraction After RealTime-Glo™ Assay

RealTime-Glo™ Assay was used to measure viability of different sizes of HEK293 3D cell spheroids followed by RNA extraction of the same samples using ReliaPrep™ RNA Tissue Miniprep System or Maxwell® 16 LEV simplyRNA Tissue Kit. The presence of RealTime-Glo™ Regent does not affect RNA yield.



9. Conclusions

A novel assay has been developed to measure viable cell number in "real time":

- Repeated kinetic luminescent measurements indicate cell growth and death over time

The real time viability assay is non-toxic and performs similar to ATP assay

- Presence of RealTime-Glo™ Reagent in culture medium does not affect cell viability, thus enabling multiplexing with a variety of other assays
- Direct comparison to ATP assay shows similar EC_{50} values for toxin

Real time detection methods provide flexibility during assay development:

- Kinetic measurements of cell viability from the same plate eliminates the need for multiple parallel plates during development and optimization of phenotypic assays
- Multiplexing real time assay methods can provide an internal control to verify viable cell number simultaneously with a variety of other phenotypic assays

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