Continuous Real-Time Measurement of Live and Dead Cells in Culture Over Multiple Days

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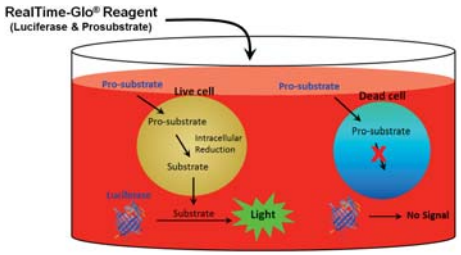
Abstract # 5446

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. Dead cells are measured by adding a novel non-toxic, non-permeable DNA-binding dye. Viable cells exclude the dye while dead cells take up the dye, which becomes fluorescent upon binding to DNA. Both assays are non-toxic to cells, so viable cells remain in the sample well following measurement of the live or dead signals. In addition to providing real time kinetic measurements that are valuable for assay development and characterization activities, multiplexing with other assays (e.g. apoptosis, oxidative stress markers or RNA extraction) provides a time saving approach and statistical advantage inherent in taking measurements from the same sample of cells.

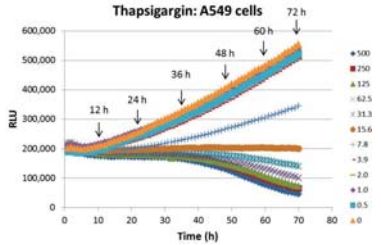
2. Real-Time Assay to Measure Live Cells

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



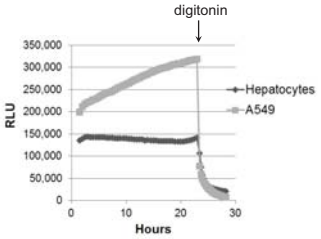
3. Three-Day Time Course Measuring Live Cell Number

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.

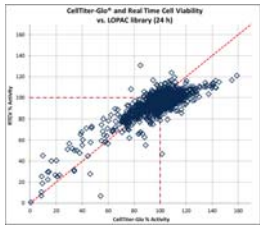


4. Loss of "Real Time" Signal Upon Cell Death & Correlation with ATP Assay Results

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

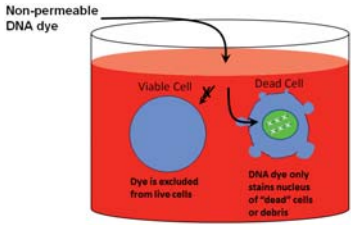


There is good correlation between the RealTime-Glo™ and CellTiter-Glo® ATP Assays from screening LOPAC library.



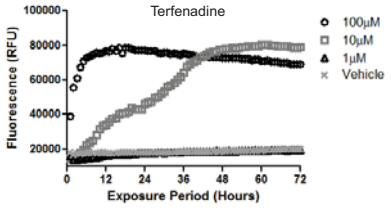
5. Real-Time Assay to Measure Dead Cells

CellTox-Green™ DNA binding dye is added directly to cell culture. The dye is non-toxic and not permeable to live cells; but penetrates dead cells to stain DNA. Fluorescence is proportional to the number of dead cells that accumulate over time in culture.



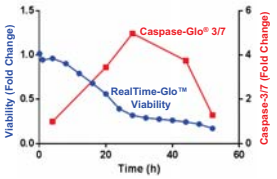
6. Three-Day Time Course Measuring Dead Cells

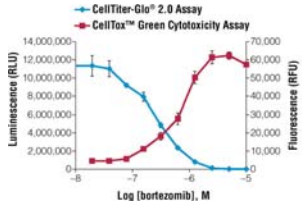
Increasing fluorescence over time indicates an increase in the number of dead cells. HepG2 cells were treated with various doses of Terfenadine. CellTox™ Green Dye was added and fluorescence was measured every hour for 3 days.



7. Multiplexing Examples Using Real Time Assays

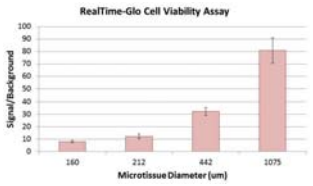
(**Left**) THP1 cells were grown in medium containing the RealTime-Glo™ Assay reagents and treated with 1 µM doxorubicin. Cell viability was monitored every 4 hours and Caspase-Glo® 3/7 multiplexed at indicated times. (**Right**) CellTox-Green™ DNA dye was added to K562 cells 48 hours after exposure. Fluorescence (indicating dead cells) was measured, then CellTiter-Glo® 2.0 Reagent was added and luminescence (indicating viable cells) was measured.

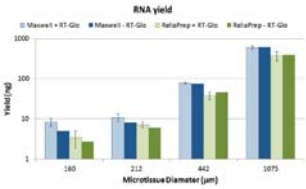




8. Multiplexing RNA Extraction After RealTime-Glo™ Assay

RealTime-Glo™ Assay was used to measure viability of different sizes of HEK293 cell spheroids followed by RNA extraction of the same samples using ReliaPrep™ RNA Tissue Miniprep System or Maxwell® 16 LEV simplyRNA Tissue Kit.





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
- Reagents are not toxic, thus cells remain viable for subsequent multiplexing assays

A non-toxic non-permeable DNA dye can measure dead cell number in real time:

- Repeated fluorescence measurements indicate appearance of dead cells over time
- DNA dye is non-toxic, thus cells remain viable for subsequent multiplexing of assays

Real time detection methods provide flexibility during assay development:

- Kinetic measurements of cell health parameters 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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