Multiplexing a Real-Time Cell Viability Assay and RNA Extraction from the Same 3D Spheroids

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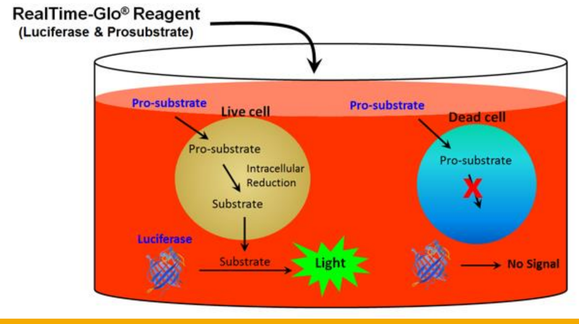
1. Introduction

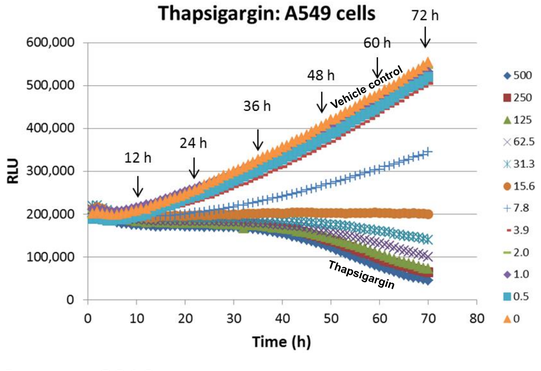
Although the implementation of 3D cell culture models is growing rapidly, there is an unmet need to establish *in vitro* assay methods to interrogate larger and more complex 3D structures. We report here the results of using a novel small molecule probe that viable cells convert into a substrate for a shrimp-derived luciferase to generate a signal proportional to the number of viable cells. The detection reagent can be incubated with cells and produce a luminescent signal for days while having minimal effect on the biology of the system. An advantage of this approach is that viable cells remain in culture which can be multiplexed with a variety of other assay chemistries such as orthogonal methods of confirming viable cell number, measuring dead cells, gene reporter assays, apoptosis assays, and extraction of high quality RNA from individual 3D spheroids. The results of RNA extraction from the same samples used for viability measurements demonstrate the presence of the real-time cell viability reagent did not have an effect on yield or integrity of the RNA. Multiplexing the real-time assay with other methods provides a more efficient experimental approach compared to evaluating replicate samples from parallel assay plates.

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 remains proportional to the number of live cells for up to 3 days.



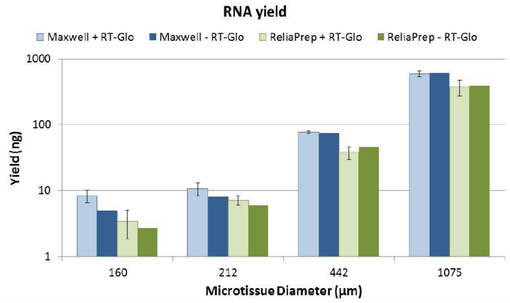
3. Three-Day Time Course Measuring Live Cell Number



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. RNA Extraction Following the RealTime-Glo™ Assay of 3D Spheroids

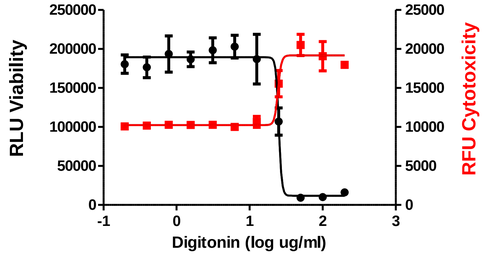
RealTime-Glo™ Assay was used to measure viability of different sizes of HEK293 cell spheroids (not shown) followed by RNA extraction of the same samples using ReliaPrep™ RNA Tissue Miniprep System or Maxwell® 16 LEV simply RNA Tissue Kit. The presence of the RealTime-Glo™ Reagent had no effect on the yield or quality RNA (RIN value).



5. Example Workflow for Multiplexing RNA Extraction After the RealTime-Glo™ Assay

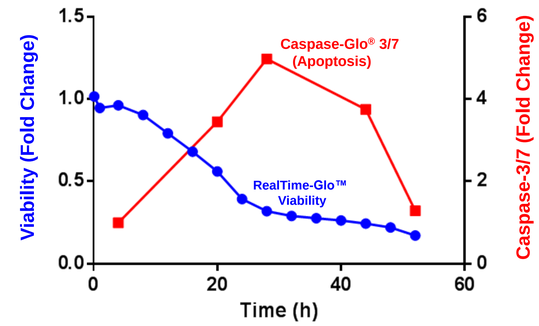
6. Multiplexing Live Cell and Dead Cell Assays

A549 cells were plated in a 384-well plate in the presence of RealTime-Glo™ Reagent and CellTox™ Green DNA binding dye. Luminescence (viable cells) and fluorescence (dead cells) were recorded 1 h post-digitonin treatment.



7. Multiplexing Real Time Live Cell & Apoptosis Assays

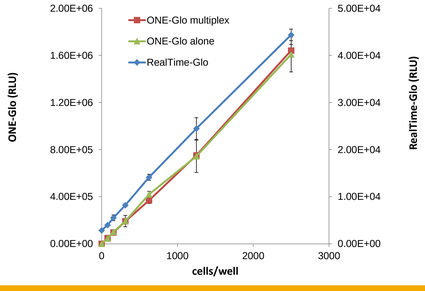
THP1 cells were grown in medium containing the RealTime-Glo™ Assay Reagent and treated 1 µM doxorubicin. Cell viability was monitored every 4 hours and Caspase-Glo® 3/7 multiplexed at indicated times to detect apoptosis.



8. Multiplexing Luminescent Cell Viability and Firefly Luciferase Reporter Assays

Luminescent signal from the RealTime-Glo™ Assay decreases immediately after cell death (not shown) enabling multiplexing of a different luciferase assay chemistry. Firefly luciferase reporter assay signal is not affected by the presence (red squares) or absence (green triangles) of RealTime-Glo™ Reagent.

Seed HEK293 cells expressing luciferase in 384 well plate
↓
Incubate overnight
↓
Add RealTime-Glo™ Reagent
↓
Incubate 2 hours
↓
Record Luminescence
↓
Add firefly luciferase reagent
↓
Incubate 10min
↓
Record luminescence



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.
- Cells remain viable enabling subsequent multiplexing of other 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 the real time cell viability assay provides an internal control to verify viable cell number simultaneously with extraction of RNA for downstream analysis of gene expression.
- The real time viability assay is compatible for multiplexing with a variety of other assay chemistries.

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