

Promega's multiplexed cell viability and apoptosis assays

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- Multiplexing homogeneous cell-based assays for analysis of different parameters from a single sample well
- Monitoring of both luminescence and fluorescence output signals from assay well
- Assay miniaturization up to 1536-well format

Introduction

Today's high-throughput screening facilities face increasing demands to generate more information from their existing compound libraries. One method of obtaining this information is to run assays sequentially, looking at one parameter followed by another in different plates. While this option may produce the desired data, the increased time and consumable costs are drawbacks. A more appealing method for data generation is to perform assays in a multiplexed format in which several parameters can be measured within the same well. This multiplexed format not only saves time and consumable cost, but also saves on the usage of valuable test compounds. This concept of assay multiplexing is demonstrated here using several cell-based assays multiplexed together. There are inherent properties to cell assays that make them attractive for multiplexed cell-based applications. Cell-based assays are especially vulnerable to variations due to differences in cell growth and metabolism that can arise from plate-to-plate. Cell culture itself is also expensive. By multiplexing assays, fewer cells are needed to acquire the same amount of data. Using the same cells for subsequent assays can also ensure more precise data. In this application note, we demonstrate the combination of several Promega cell-based assays multiplexed in both low-volume 384- and 1536-well plate formats. Table 1 + 2 highlight the assays used in this application note.

Table 1: Cell-based assays for multiplexing applications, set 1.

Assay/ Detection mode	Assay Description
CellTiter-Glo® Luminescence	Cell viability based on the quantification of ATP contained in viable cells in culture. Luminescence is directly proportional to the number of viable cells.
EnduRen™ Luminescence	<i>Renilla</i> luciferase reporter luminescence via a protected coelenterazine substrate designed to generate <i>Renilla</i> luminescence from living cells. Once inside the cell, the protective groups of the substrate are cleaved by intracellular esterases, generating coelenterazine which reacts with <i>Renilla</i> to produce light. Peak luminescence is achieved after 1.5 hours of substrate addition to cells, and signal is stable for > 24 hours.
ViviRen™ Luminescence	<i>Renilla</i> luciferase reporter luminescence via a protected coelenterazine substrate designed to generate <i>Renilla</i> luminescence from living cells. Once inside the cell, the protective groups of the substrate are cleaved by intracellular esterases, generating coelenterazine which reacts with <i>Renilla</i> to produce light. Peak luminescence is achieved after 2 minutes of substrate addition to cells, with signal half-life from 8 – 15 minutes.

Table 1: Cell-based assays for multiplexing applications, set 2.

Assay/ Detection mode	Assay Description
Caspase-Glo® 3/7 Luminescence	Activity of caspase-3 and caspase-7 in cells undergoing apoptosis via cleavage of a Z-DEVD-luciferin derivative. Luciferin reacts with luciferase, ATP and oxygen to produce light. Light output is directly proportional to caspase activity.
Apo-ONE® Fluorescence	Activity of caspase-3 and caspase-7 in cells undergoing apoptosis via cleavage of a Z-DEVD-R110 substrate. The R110 leaving group becomes intensely fluorescent. R110 fluorescence is directly proportional to caspase activity.
CellTiter-Blue® Fluorescence	Cell viability based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resorufin). Viable cells retain the ability to reduce resazurin into resorufin, whereas non-viable cells lose this ability. Fluorescence is directly proportional to the number of viable cells.

Materials & Methods

- Dedicated Promega assay kits
- Corning low-volume 384-well plates
- Corning 1536-well plates

Multiplexing Cell Viability and Apoptosis Assays

Promega's fluorescent CellTiter-Blue® cell viability assay was multiplexed with either the luminescent Caspase-Glo® 3/7 assay, or the fluorescent Apo-ONE® assay.

Multiplexing Luciferase Reporter and Cell Viability Assays

Promega's luminescent CellTiter-Glo® assay was multiplexed with either the luminescent EnduRen™ Live Cell Substrate, or the luminescent ViviRen™ Live Cell Substrate.

Assay Miniaturization to 1536-well format

For the 1536-well assay format, a density of 4,000 stably transfected cells per well was plated with the Deerc Fluidics Equator. The multiplex protocols were performed identically to the low-volume 384-well (more detailed information on the methods can be found at www.bmglabtech.com).

Results & Discussion

Multiplexing Cell Viability and Apoptosis Assays

Figures 1 and 2 show the results of two experiments to determine the method of cell death caused by different concentrations of anti-FAS antibody in Jurkat cells. The two experiments measured two different endpoints:



reduction of a resazurin dye as an indicator of viable cells and caspase activity as a marker for apoptotic cells. The data show that with increasing concentration of anti-FAS antibody, an increase in caspase-3/7 activity, with a corresponding decrease in cell viability, is observed.

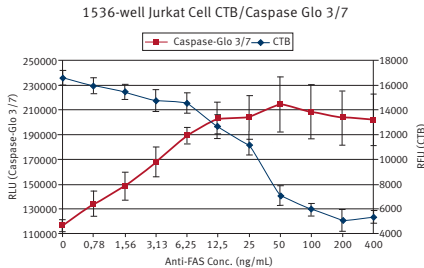


Fig. 1: Sequential multiplexing of a fluorescent cell viability [CellTiter-Blue®] with a luminescent apoptosis assay [Caspase-Glo® 3/7] in 1536-well format.

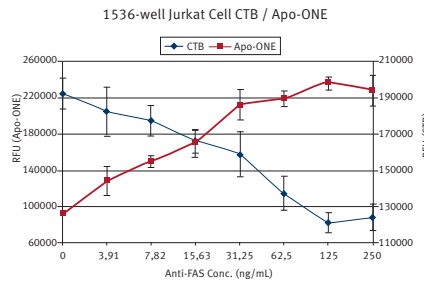


Fig. 2: Sequential multiplexing of a fluorescent cell viability assay [CellTiter-Blue®] with a fluorescent apoptosis assay [Apo-ONE®] in 1536-well format.

The results suggest that the cell population studied is less viable over the range of treatment due to an increase in apoptosis, as opposed to necrosis. For all cell viability and apoptosis multiplexing combinations, results in 1536-well format are comparable to results in 384-well format.

Luciferase Reporter and Cell Viability Assays

To correlate Renilla luciferase reporter gene signal for cell viability, Promega's CellTiter-Glo® assay was multiplexed with either the EnduRen™ Live Cell Substrate or the ViviRen™ Live Cell Substrate. After luminescence reporter signal determination, the CellTiter-Glo® reagent was added at each measurement point to inactivate Renilla luminescence and initiate ATP-dependent luminescence, which was recorded to measure the cell viability (Fig. 3 and 4).

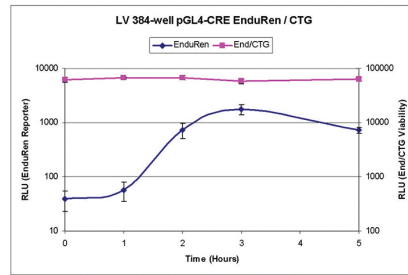


Fig. 3: Coupled Renilla reporter activity with cell viability in 384-well format. The kinetic profile of a Renilla reporter gene is correlated with the overall cell number.

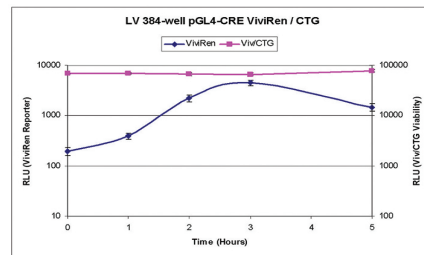
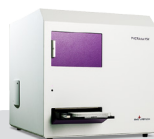


Fig. 4: Coupled Renilla reporter activity with cell viability. Renilla luminescence is first recorded. CellTiter-Glo® Reagent is added, followed by luminescence reading in 384-well format.

Using live cell reporter substrates, it is possible to track the response of a Renilla reporter in real time. Including a cell viability assay allows one to correlate reporter response with overall cell number.

Conclusion

Data from miniaturized assays in 1536-well format are comparable to those run in low volume 384, indicating that smaller assay volume does not compromise the results obtained in higher density formats.



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