

Promega's Multiplexed Cell Viability and Apoptosis Assays performed on the PHERAstar

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- Correlated measurement of an apoptosis marker (or caspase activity) and cell viability
- Monitoring of both luminescence and fluorescence output signals from one 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 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. The BMG LABTECH PHERAstar microplate reader is used to record both luminescence and fluorescence, depending on the multiplex combination. Table 1 highlights the assays used in this application.

Table 1: Cell-based assays for multiplexing applications

Assay	Readout mode	Parameter measured
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 and Methods

- Jurkat cells
- Anti-FAS monoclonal antibody
- Corning Low-volume 384-well plates, catalog no. 3673
- Corning 1536-well plates, catalog no. 3937
- Promega Caspase-Glo® 3/7 Assay System, catalog no. G8091*
- Promega CellTiter-Blue® Cell Viability Assay, catalog no. G8080
- Promega Apo-ONE® Homogeneous Caspase 3/7 Assay, catalog no. G7790
- Deerc Fluidics™ Equator™ HTS low-volume liquid dispenser
- BMG LABTECH PHERAstar (figure 1)
- BMG LABTECH PHERAstar luminescence optic module
- BMG LABTECH PHERAstar fluorescence optic module (Ex: 485 nm; Em: 520 nm)
- BMG LABTECH PHERAstar fluorescence optic module (Ex: 540 nm; Em: 590 nm)



Fig. 1: BMG LABTECH's multimode plate reader PHERAstar

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. The experimental set-up was similar for each assay combination.

For the low-volume 384 assay format, a density of 10,000 Jurkat cells per well was plated with the Deerc Fluidics Equator. Next, a range of anti-FAS monoclonal antibody was added to the plate, with the final concentration per well ranging from 400 ng/mL down to 0 ng/mL of antibody. The plates were then incubated at 37°C / 5% CO₂ for a total of 5 hours to induce apoptosis. 3 hours into the 5 hour treatment, CellTiter-Blue® reagent was added to each well with the Equator (note: for plates later receiving Caspase-Glo® 3/7 reagent, CellTiter-Blue® was diluted 1:4 in 1X PBS before addition to the assay plate). After the CellTiter-Blue® addition, plates were incubated for 2 hours at 37°C / 5% CO₂. When the 5 hour incubation with anti-FAS antibody was complete, fluorescence was recorded at excitation 540 nm and emission of 590 nm with the PHERAstar. The caspase reagents were then added to the plates with the Equator. Apo-ONE® was added to one plate containing CellTiter-Blue® reagent, and the plate was incu-

bated at room temperature for 1 hour, followed by fluorescence reading with the PHERAstar at excitation 485 nm and emission of 520 nm. Caspase-Glo[®] reagent was added to the plate receiving the diluted CellTiter-Blue[®] reagent, and incubated for 1 hour at room temperature. Luminescence was then recorded with the PHERAstar. For the 1536-well assay format, a density of 4,000 cells per well was plated with the Deerac Fluidics Equator. The remaining multiplex protocols were performed identically to the low-volume 384 protocols listed above.

Results and Discussion

Promega's CellTiter-Blue[®] assay multiplexed with either Caspase-Glo[®] 3/7 (figure 2) or Apo-ONE[®] assay was prepared in both low-volume 384- and 1536-well format (figure 3). Cell viability and apoptosis were sequentially measured with BMG LABTECH's PHERAstar in both luminescence and fluorescence modes, depending on the multiplex combination. Jurkat cells were treated with various concentrations of anti-FAS monoclonal antibody for 5 hours to induce apoptosis. Cell viability was determined by adding CellTiter-Blue[®] reagent to each well after drug addition and incubating for two hours before recording fluorescence (Ex: 540 nm; Em: 590 nm). The caspase activity was then measured by adding either Caspase-Glo[®] 3/7 or Apo-ONE[®] reagent and incubating for an additional hour prior to recording luminescence or fluorescence (Ex: 485 nm; Em: 520 nm) respectively.

Figures 2 and 3 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.

Cell Viability and Apoptosis Assay Multiplex combinations

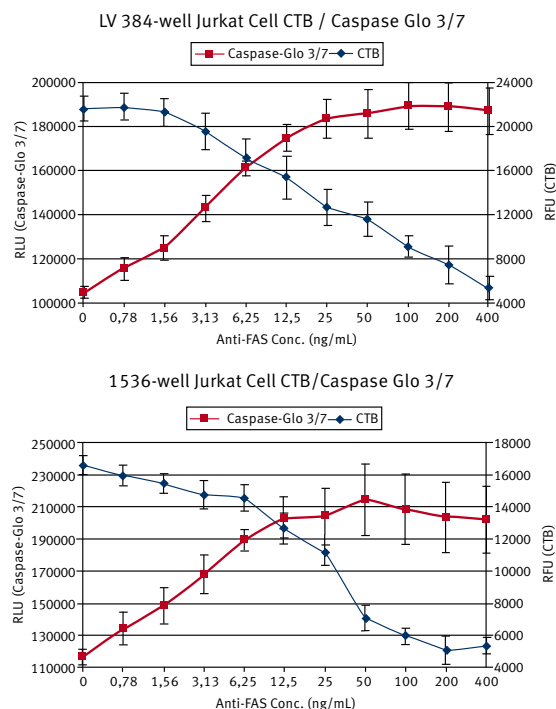


Fig. 2: Sequential multiplexing of a fluorescent cell viability assay (CellTiter-Blue[®]) with a luminescent apoptosis assay (Caspase-Glo[®] 3/7). The CellTiter-Blue[®] reagent is added first to the well, followed by fluorescence recording. The Caspase-Glo[®] 3/7 reagent is added second, followed by recording of luminescence. Both readings were performed with the PHERAstar.

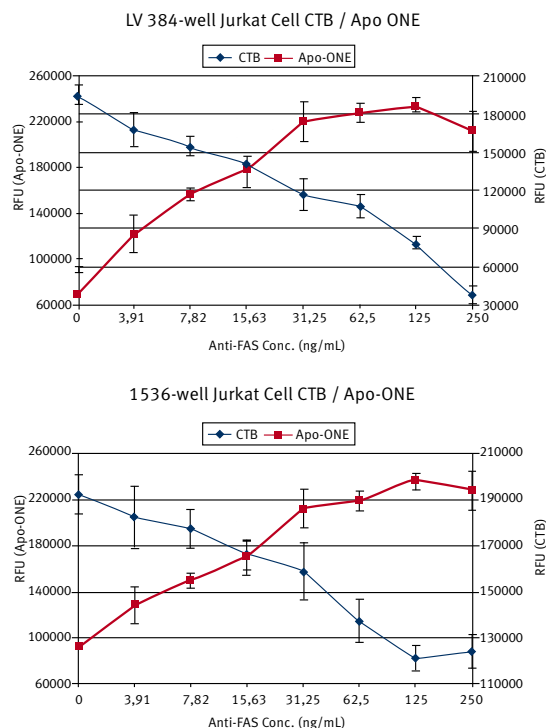


Fig. 3: Sequential multiplexing of a fluorescent cell viability assay (CellTiter-Blue[®]) with a fluorescent apoptosis assay (Apo-ONE[®]). The CellTiter-Blue[®] reagent is added first, followed by fluorescence recording. The Apo-ONE[®] reagent is added second, followed by fluorescence recording. Both readings were performed with the PHERAstar.

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 format are comparable to results in 384-well format.

Conclusion

Each of the experiments shown here highlights the ability to perform different assays within the same assay well. Using a non-lytic assay first, such as the CellTiter-Blue[®] assay used here, allows for the sequential multiplexing of several different reagents within the same well. Multiplexed cell-based assays allow for multiple parameters to be measured within the same well. One example of this is to determine the method of cell death following a certain treatment protocol. By performing two assays within the same well, information on mode of action of drugs could be obtained faster and with less consumable usage. 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. The data generated here also showcases the ability of BMG LABTECH's multifunctional PHERAstar to record multiple output signals from the same assay well.

* See Promega's website for patent marking information
www.promega.com

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