# Promega's Multiplexed Luciferase Reporter and Cell Viability Assays performed on the PHERAstar

Tracy Worzella and Brad Larson
Promega Corporation, Madison, WI, USA

Application Note 139 Rev. 7/2006

- Multiplexing homogeneous cell-based assays for analysis of different parameters from a single sample well
- Monitoring of reporter gene activity and cell viability
- 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 useage 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. The BMG LABTECH PHERAstar microplate reader is used to record luminescence. Table 1 highlights the assays used in this application.

**Table 1:** Cell-based assays for multiplexing applications

| Assay | Readout mode | Parameter measured |
|---|---|---|
| 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 | *Renilla* luciferase reporter luminescence via a protected coelenterazine substrate designed to generate *Renilla* luminescence from living cells. Once inside the cell, the protective groups of the substrate are cleaved by intracellular esterases, generating coelenterazine which reacts with *Renilla* 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 | *Renilla* luciferase reporter luminescence via a protected coelenterazine substrate designed to generate *Renilla* luminescence from living cells. Once inside the cell, the protective groups of the substrate are cleaved by intracellular esterases, generating coelenterazine which reacts with *Renilla* to produce light. Peak luminescence is achieved after 2 minutes of substrate addition to cells, with signal half-life from 8 – 15 minutes. |

## Materials and Methods

- HEK293 cells stably transfected with a CRE/CL1 hPEST *Renilla* construct
- Isoproterenol
- Corning Low-volume 384-well plates, catalog no. 3673
- Corning 1536-well plates, catalog no. 3937
- Promega CellTiter-Glo® Luminescent Cell Viability Assay, catalog no. G7570*
- Promega EnduRen™ Live Cell Substrate, catalog no. E6482*
- Promega ViviRen™ Live Cell Substrate, catalog no. E6492*
- Deerac Fluidics™ Equator™ HTS low-volume liquid dispenser
- BMG LABTECH PHERAstar (figure 1)
- BMG LABTECH PHERAstar luminescence optic module


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

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

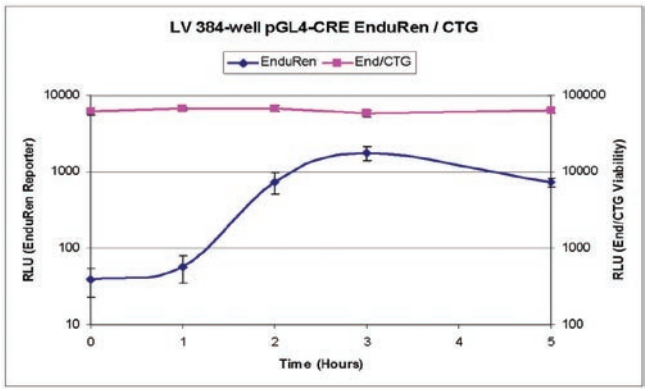
For the low-volume 384 assay format, a density of 12,000 stably transfected HEK 293 cells per well was plated with the Deerac Fluidics Equator. Cells were allowed to attach for 10 hours at 37°C / 10% $CO_2$. EnduRen™ Live Cell Substrate was added by the Equator to one plate of cells at a final concentration of 60 µM and allowed to incubate with the cells for 2 hours at 37°C / 10% $CO_2$. 10 µM of isoproterenol was added to all test plates to induce CRE. Induction was monitored at 0, 1-, 2-, 3-, and 5-hour time points. At each time point, ViviRen™ substrate was added by the Equator at a final concentration of 60 µM, luminescence reporter signal was recorded, followed by addition of the CellTiter-Glo® reagent by the Equator. Luminescence was recorded a second time to measure ATP content and cell number.

For the 1536-well assay format, a density of 4,000 stably transfected HEK 293 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

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. Both multiplexed assay combinations were prepared in both low-volume 384- and 1536-well format and *Renilla* expression and cell viability were sequentially measured with BMG LABTECH's PHERAstar in luminsecence mode (figure 2 and figure 3).

HEK 293 cells were treated with isoproterenol for 5 hours to induce *Renilla* reporter expression and a correlated kinetic profile of the reporter signal and cell viability were recorded over this time. The EnduRen™ Live Cell Substrate was added 2 hours before CRE induction and allowed to incubate with the cells. The ViviRen™ Live Cell Substrate was added directly before each measurement point. 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.



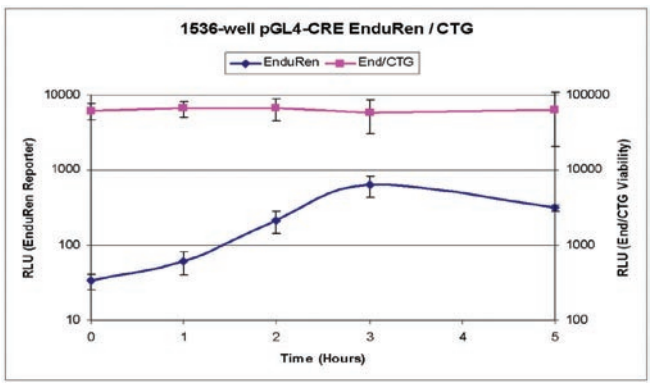
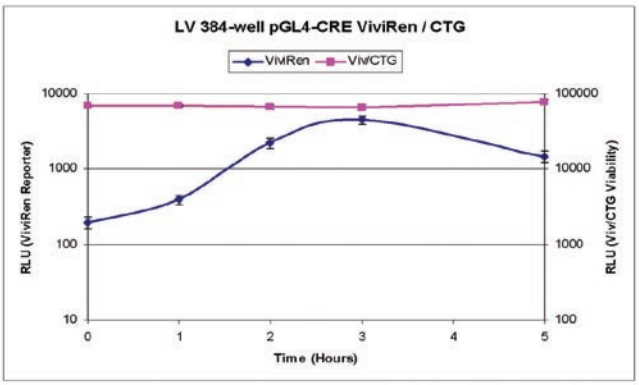




**Fig. 2:** Coupled *Renilla* reporter activity with cell viability. *Renilla* luminescence is first recorded with the PHERAstar. CellTiter-Glo® Reagent is added, followed by luminescence reading. The kinetic profile of a *Renilla* reporter gene is correlated with the overall cell number.

When making correlations between experimental conditions and the expression of a reporter gene, other events associated with cell physiology may affect reporter gene expression. Using live cell reporter substrates, it is possible to track the response of a *Renilla* reporter in real time by measuring luminescent *Renilla* reporter activity. Including a cell viability assay allows one to correlate reporter response with overall cell number. For this application, the *Renilla* reporter expression was optimal at 3 hours of treatment with 10 µM isoproterenol in both assay formats tested, regardless of which substrate was used. Cell viability did not change over the time tested, indicating that changes in reporter response were due to the treatment and not changes in cell number. Using the same experimental conditions, the results also show the increased luminescence generated by the ViviRen™ substrate over the EnduRen™



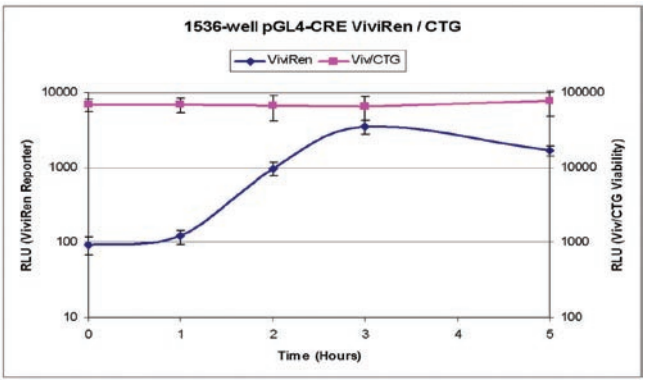




**Fig. 3:** Coupled *Renilla* reporter activity with cell viability. *Renilla* luminescence is first recorded with the PHERAstar. CellTiter-Glo® Reagent is added, followed by luminescence reading. Compared to EnduRen™, the ViviRen™ substrate produces higher overall luminescence signal. The kinetic profile of a *Renilla* reporter gene is correlated with the overall cell number.

substrate. The ViviRen™ substrate would be a good option when using cell lines that are weakly expressing *Renilla* luciferase, or for instances when fewer cells are being used as is the case for 1536-well format. Low volume 384 results also correlate with those in 1536, suggesting that miniaturization of these assays does not compromise data quality.

## Conclusion

Multiplexed cell-based assays allow for multiple parameters to be measured within the same well. With reporter assays, the expression of a luciferase reporter can be greatly affected by the overall health of the cell. By multiplexing a reporter assay with a cell viability assay, it is possible to determine if reporter response variations are due to changes in cell number and health. Depending on the *Renilla* expression levels or the number of cells being used, the EnduRen™ and ViviRen™ Live Cell Substrates provide options for the customer looking to observe real-time reporter kinetics. The sensitivity of the PHERAstar allowed for luminescence detection from both reporter substrates. The data generated here also showcases the ability of BMG LABTECH's PHERAstar to record luminescence from microplates of different well densities up to 1536-wells.

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


| | | |
|---|---|---|
| **Germany:** | **BMG LABTECH GmbH** | **Tel: +49 781 96968-0** |
| Australia: | BMG LABTECH Pty. Ltd. | Tel: +61 3 59734744 |
| France: | BMG LABTECH SARL | Tel: +33 1 48 86 20 20 |
| Japan: | BMG LABTECH JAPAN Ltd. | Tel: +81 48 647 7217 |
| UK: | BMG LABTECH Ltd. | Tel: +44 1296 336650 |
| USA: | BMG LABTECH Inc. | Tel: +1 919 806 1735 |
| **Internet:** | **www.bmglabtech.com** | **info@bmglabtech.com** |