Monitor Cell Viability and Reporter Gene Expression in the Same Well with the...

Monitor Cell Viability and Reporter Gene Expression in the Same Well with the One-Glo™+Tox Luciferase Reporter and Cell Viability Assay

ABSTRACT

We used the ONE-Glo™+Tox Luciferase Reporter and Cell Viability Assay to demonstrate the importance of evaluating reporter gene expression in the context of cell health. Ionomycin titration results exhibit decreased light output from firefly luciferase at high concentrations, while the cell viability assay reveals cytotoxicity as the reason for the decrease in luciferase expression.

Tracy Worzella, Andrew Niles, Mike Valley, Michael Bjerke and Kevin Kopish. Promega Corporation

Publication Date: 2012

Introduction

The manipulation of an experimental system for transfection optimization or evaluation of drug effects on a cellular pathway often results in toxicity that can affect the interpretation of the reporter gene assay. To control for this, parallel viability assays are often performed alongside the reporter assay. This approach helps to identify effects on the reporter system versus cytotoxic artifacts, but requires the use of more cells, plastics and reagent consumables. In addition, performing reporter and viability assays in separate plates increases the chance of acquiring skewed data due to processing errors.

The ONE-Glo™+Tox Luciferase Reporter and Cell Viability Assay (Cat.# E7110, E7120) allows performance of both reporter and viability assays in the same well, providing a multiplexed, sequential-addition method for evaluating firefly luciferase expression in the context of cell health. The first assay is a non-lytic fluorescent assay (CellTiter-Fluor™) that measures a protease biomarker present in live cells. Live-cell protease cleaves a non-fluorescent GF-AFC substrate, generating free AFC that is measured with a fluorescence plate reader. The fluorescence generated is proportional to cell viability and cell number. The ONE-Glo™ Luciferase Assay Reagent is then added, lysing the cells and quantifying reporter gene expression in a firefly luciferase reaction. Light output is quantified with a luminometer.

Here we demonstrate the utility of the ONE-Glo™+Tox assay in an automated application using a cell model stably expressing firefly luciferase under the control of the NFAT response element, a prototype Gilson Bio 1000 liquid handler for assay setup and reagent addition, and the GloMax®- Multi+ microplate reader (Cat.# E9032) for detection.

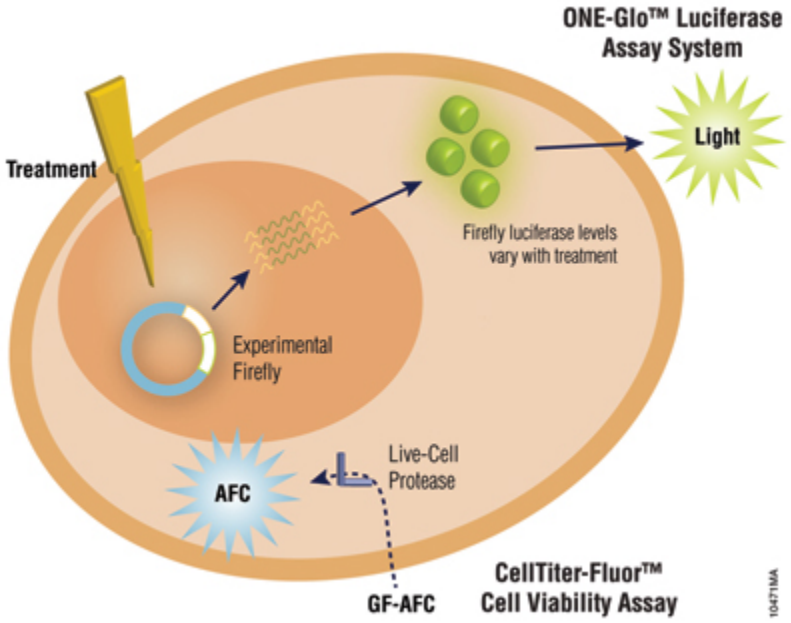

Figure 1. Schematic of the ONE-Glo™+Tox Luciferase Reporter and Cell Viability Assay.

Method

1. A cell suspension was prepared at a density of 2x10$_5$ cells/ml in assay medium (RPMI 1640 + 10% FBS).
2. A 200 µM working stock of ionomycin (Sigma, Cat.# I0634) was prepared in assay medium diluent containing 2 nM PMA (Sigma, Cat.# P8139).
3. Two hundred microliters of the ionomycin working stock was manually pipetted to column one of a 96-well dilution plate (Corning, Cat.# 3370), which was then placed onto the deck of the Gilson Bio 1000.
4. The Bio 1000 transferred 100 µl of diluent to the dilution plate and subsequently performed a serial 1:2 titration of ionomycin across the plate. Fifty microliters of the dilution series was transferred to a white 96-well assay plate (Corning, Cat.# 3917).
5. Fifty microliters of the cell suspension was then transferred with the Bio 1000 to the assay plate. The assay plate was incubated at 37°C/5% CO_2 for 5 hours to induce firefly luciferase expression.
   5X CellTiter-Fluor™ Reagent was prepared by combining 25 µl of GF-AFC substrate with 5 ml of Assay Buffer. Twenty microliters of 5X CellTiter-Fluor™ was added to each well of the assay plate, followed by a 30-second plate shake and 30-minute incubation at 37°C/5% CO_2. Fluorescence was recorded using the AFC filter module.
6. One hundred microliters of reconstituted ONE-Glo™ Reagent was added to the plate, followed by an off-line plate shake and 3-minute incubation at room temperature. Luminescence was recorded.

Results

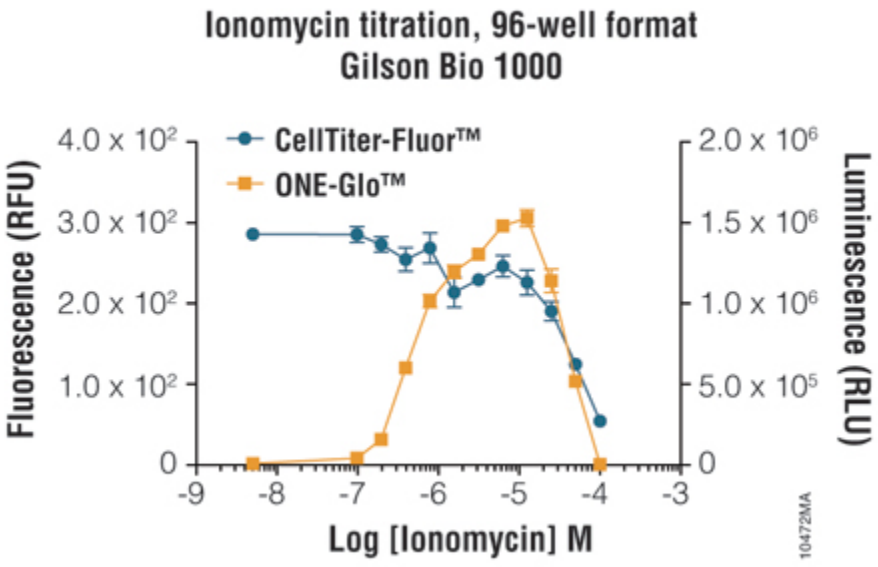

Figure 2. Luciferase reporter expression (ONE-Glo™ Assay) and cell viability (CellTiter-Fluor™ Assay) at increasing ionomycin concentration.

At specific concentrations, ionomycin and PMA work cooperatively to stimulate NFAT-dependent firefly luciferase gene expression. However, higher concentrations of ionomycin result in cytotoxicity, which is shown as a decrease in viability (CellTiter-Fluor™ Assay). A decrease in reporter expression (ONE-Glo™ Assay) is also observed due to the increase in cytotoxicity. More dilute concentrations of ionomycin show a dose-dependent effect on firefly luciferase expression, with no effect on cell health.

Conclusion

The ONE-Glo™+Tox Luciferase Reporter and Cell Viability Assay was used in an ionomyocin dose response experiment to demonstrate the importance of evaluating reporter gene expression in the context of cell health. A decrease in firefly reporter gene expression, with corresponding decrease in viability, was observed at higher concentrations of drug treatment. The reporter gene results alone would lead one to conclude that certain concentrations of ionomycin were not effective in inducing firefly luciferase expression. The cell viability readout confirms that decreased reporter expression at higher concentrations is likely a result of global cytotoxicity due to treatment. The multiplex described here can help identify false positive wells in loss-of-signal assay scenarios where a knockdown in activity is the desired outcome. In this case, the viability control will help discern effects on the experimental system versus effects on cell health. Gain-of-signal systems can also realize the benefits of this multiplex. In that scenario, the viability control can help identify false positives due to over-dispensed cells versus true induction of luciferase expression.

HOW TO CITE THIS ARTICLE

Scientific Style and Format, 7th edition, 2006

Worzella, T., Niles, A., Valley, M., Bjerke, M., and Kopish, K. Monitor Cell Viability and Reporter Gene Expression in the Same Well with the One-Glo™+Tox Luciferase Reporter and Cell Viability Assay. [Internet] 2012. [cited: year, month, date]. Available from: http://www.promega.com/resources/pubhub/monitor-cell-viability-and-reporter-gene-expression-in-the-same-well/

American Medical Association, Manual of Style, 10th edition, 2007

Worzella, T., Niles, A., Valley, M., Bjerke, M., and Kopish, K. Monitor Cell Viability and Reporter Gene Expression in the Same Well with the One-Glo™+Tox Luciferase Reporter and Cell Viability Assay. Promega Corporation Web site. http://www.promega.com/resources/pubhub/monitor-cell-viability-and-reporter-gene-expression-in-the-same-well/ Updated 2012. Accessed Month Day, Year.