

APPLICATION NOTE

Measuring luciferase expression using the SpectraMax Glo Steady-Luc Reporter Assay Kit

Introduction

The use of gene reporters such as luciferase permits highly sensitive and nondestructive monitoring of gene expression. Firefly luciferase, a 61 kD monomeric protein, is especially attractive to many researchers because of its high sensitivity, wide linear detection range and extremely low background due to the absence of endogenous luminescent activity in mammalian cells. For the increasingly popular assay using luminescence microplate readers, the high-throughput compatible “glow” assay is often preferred for batch processing of multiple plates. In this application note, we demonstrate the measurement of luciferase expression in CHO-K1 cells using the SpectraMax® Glo Steady-Luc™ Reporter Assay Kit, which affords long-lasting luminescence signals. This assay kit is optimized for the SpectraMax® i3x Multimode Microplate Reader with a preconfigured protocol in SoftMax® Pro Software for rapid data analysis.

Materials

- SpectraMax Glo Steady-Luc Reporter Assay Kit, Molecular Devices (Explorer kit #R8352 or Bulk kit #R8353)
- Competitor kit: Steady-Glo Luciferase Assay System, Promega (Cat# E2510)
- SpectraMax i3x Microplate Reader, Molecular Devices (Cat# i3X)
- 96-well white walled clear bottom plates, Costar (Cat# 3903)
- Purified luciferase, Promega (Cat# E1701)
- 384-well solid white plates, Greiner (Cat# 655075)
- CHO-K1 cells, ATCC (Cat# CCL-61)

- Complete Growth Media
 - Ham's F12 Medium, Life Technologies (Cat# 11765-54)
 - Fetal Bovine Serum, Gemini (Cat# 100-106)
 - Penicillin/Streptomycin, Life Technologies (Cat# 15070-063)
- 0.05% Trypsin EDTA, Life Technologies (Cat# 25300-054)
- pGL4 Firefly Luciferase Vector, Promega (Cat# E6681)
- pGL3 Control Vector, Promega (Cat# 1741)
- FuGeneHD Transfection Reagent, Promega (Cat# E2311)

Methods

The SpectraMax Glo Steady-Luc Reporter assay has a simplified workflow. The working solution is mixed at 1:1 ratio with the medium in each well of the microplate, where cells expressing luciferase are plated. The plate is then covered and mixed to allow complete cell lysis before the luminescent signals are read on the SpectraMax i3x Multimode Microplate Reader (Figure 1). The data acquisition and analysis is easily streamlined when using the preconfigured protocol in Softmax Pro Software.

Benefits

- **Glow-based luminescence** provides an extended signal time window enabling batch processing of plates in screening assays
- **Optimized for SpectraMax** luminescence microplate readers
- **Simplified data acquisition and analysis** with preconfigured protocol in SoftMax Pro Software

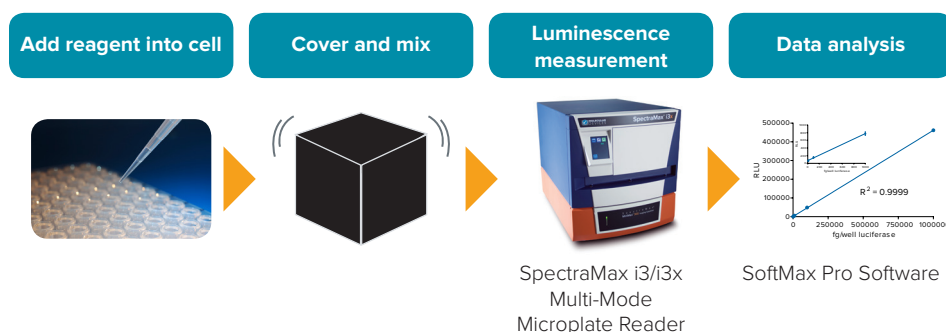


Figure 1. SpectraMax Glo Steady-Luc Reporter Assay Kit workflow.

Luciferase standard curve

To identify the linear detection range of this assay, a standard curve was constructed to measure the RLU as a function of luciferase concentration. All components of the SpectraMax Glo Steady-Luc Reporter Assay Kit were first allowed to equilibrate to room temperature. SpectraMax Glo Steady-Luc working solution was made by adding D-Luciferin to the Steady-Luc Assay buffer in a 1 mg to 4 mL ratio. A ten-fold serial dilution of purified luciferase in PBS with 0.01% BSA starting at a concentration of 1×10^6 fg/well was prepared, and 25 μ L of each concentration was added in triplicate to a 384-well solid white plate. Afterwards, 25 μ L SpectraMax Glo Steady-Luc working solution was added to the wells containing luciferase and controls. The plate was shaken and incubated in the dark for 10 minutes. Using the SpectraMax i3x reader, the preconfigured SpectraMax Glo Steady-Luc Reporter assay protocol in SoftMax Pro Software was used to measure luminescence. After detection, the protocol automatically generates a curve of the data.

Transfection and cell-dilution assay

Part of assay development for a reporter gene assay is to optimize the cell number and amount of plasmid necessary to give a robust signal for screening. A cell dilution assay was performed with two different amounts of pGL4.13 firefly luciferase starting in 6-well plates. CHO-K1 cells were transiently transfected with 1.7 μ g or 0.8 μ g per well of pGL4.13[luc2/SV40] vector, which encodes the luciferase gene *luc2* under control of the SV40 early enhancer/promoter, or pGL3-Basic (control) vector, which lacked promoter and enhancer sequences. After 24 hours, cells were trypsinized and seeded at 100 μ L/well into a 96-well plate starting at 30,000 cells/well with subsequent 2-fold dilutions. 100 μ L/well Steady-Luc working solution was added to the wells. The plate was covered to protect the reagents from light and mixed using an orbital shaker. After 10 minutes, luminescence was measured and recorded on the SpectraMax i3x reader using the preconfigured SpectraMax Glo Steady-Luc Reporter assay protocol. The preconfigured protocol is found in the Reporter Assays section under the

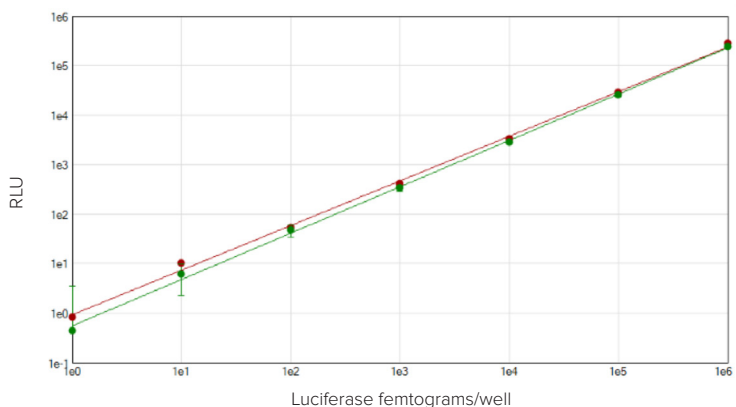


Figure 2. Luciferase standard curve in 384-well format. Linear results from a 10-fold dilution of purified luciferase in either the SpectraMax Glo Steady-Luc Reporter assay (Green) ($R^2 = 0.999$) or a competitor assay (Red) ($R^2 = 0.998$). In both assays the LLD = 5 femtogram/well.

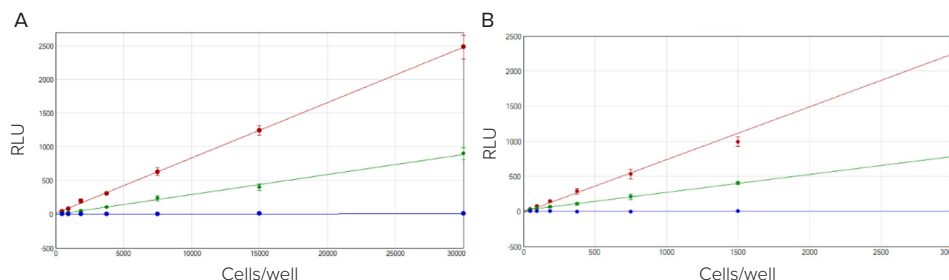


Figure 3. Measurement of luciferase in transfected cells. 90% confluent CHO-K1 cells were transfected with either 1.7 μ g pGL4 (Firefly) (Red), 0.8 μ g pGL4 (Firefly) (Green), or pGL3 (control) luciferase plasmids (Blue). **(A)** SpectraMax Glo Steady-Luc Reporter assay ($R^2 > 0.995$ at 0.8 μ g plasmid) and **(B)** Competitor assay ($R^2 = 0.999$ at 0.8 μ g plasmid). Both kits detected the lowest number of cells/well in the assay (468 cells/well).

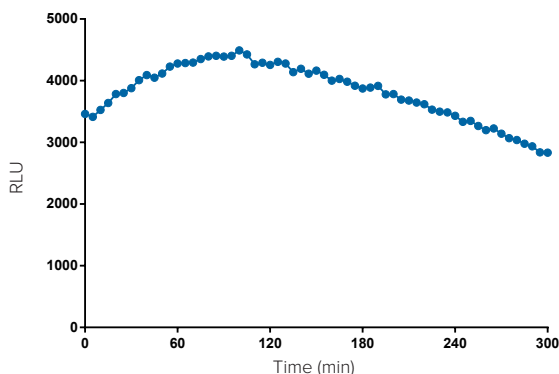


Figure 4. Luciferase signal in CHO-K1 cells over time. CHO-K1 cells were transfected with luciferase. On the day of the assay, the plate was equilibrated to room temperature, and then 100 μ L reconstituted Steady-Luc working solution containing D-Luciferin was added to each well. The plate was covered and mixed on an orbital shaker for five minutes and then placed in a SpectraMax i3x reader and mixed. Luminescence was read every five minutes for five hours with three seconds of orbital shaking before each read.

Screening applications

A reporter gene assay screen was simulated by performing multiple reads of a plate of pGI4 firefly luciferase transfected cells using the SpectraMax Glo Steady-Luc Reporter Assay Kit. Cells were transfected as previously described and plated at 30,000 cells per well in a 96-well plate and grown overnight. On the day of the assay, the plate was equilibrated to room temperature, and then 100 μ L reconstituted Steady-Luc working solution containing D-Luciferin was added to each well. The plate was covered and mixed on an orbital shaker for five minutes and then placed in a SpectraMax i3x reader and mixed. Luminescence was read every five minutes for a total of five hours with three seconds of orbital shaking before each read.

Results

Luciferase standard curve

For comparison, the standard curve to identify the detection range of the assay was also performed with a competitor's glow luciferase assay. In both cases, the assays identified a linear relationship between luminescence and luciferase concentration (Figure 2). The R^2 values for luciferase were 0.998 using the SpectraMax Glo Steady-Luc Reporter Assay Kit and 0.999 using the competitor assay kit. A paired t-test of normalized data indicates equivalent results at each concentration ($P < 0.05$). Calculations were performed using GraphPad Prism. Using both kits, the Lower Limit of Detection (LLD) of luciferase was 5 femtograms/well in 384-well format.

Contact Us

Phone: +1-800-635-5577
Web: www.moleculardevices.com
Email: info@moldev.com

Check our website for a current listing of worldwide distributors.

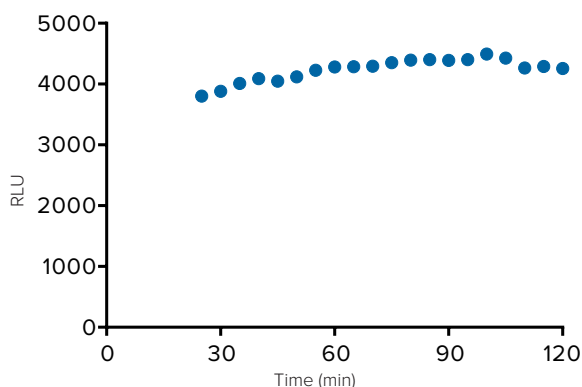


Figure 5. Screening applications. Raw data from luciferase transfected CHO-K1 cells in the SpectraMax Glo Steady-Luc assay. The average signal from the luciferase transfected cells is plotted as an example of batch processing during a screening assay in which a plate is read every five minutes. On screening plates, background controls are used to normalize data so that comparisons can be compared across many plates.

Measurement of luciferase in transfected cells

Titration of CHO-K1 cells transiently transfected with luciferase starting at 30,000 cells/well was pipetted into the wells followed by Steady-Luc Reporter working solution. Shown in Figure 3, both the SpectraMax Glo Steady-Luc Reporter assay and competitor glow luciferase assay demonstrated a linear relationship between cell number and luminescence. For cells that received 0.8 μ g of pGL4.13 luciferase vector, $R^2 = 0.995$ using the SpectraMax Glo Steady-Luc Reporter Assay Kit and $R^2 > 0.999$ using the competitor assay kit. Both kits detected the lowest dilution of cells tested in the assay, 468 cells/well.

Screening applications

The SpectraMax Glo Steady-Luc Reporter assay is a glow-based luminescence assay that provides an extended signal time window (Figure 4). A plate with luciferase expressing CHO-K1 cells at 30,000 cell/well is read every five minutes to simulate batch processing of screening plates. At five hours, the signal is within 20% of the initial value. On each individual plate in a reporter assay screen, a control for

normalization of data to background is included so that data can be compared across many plates. As demonstrated in Figure 5, the signal stays within 15% across 20 plates, suggesting the feasibility of running 20 plates in a 90 minute period with this assay.

Conclusion

Luciferase-based reporter assays using luminescence microplate readers have become increasingly popular for high-throughput analysis of chemical biology and drug discovery applications. The SpectraMax Glo Steady-Luc Reporter assay kit allows for sensitive quantification of firefly luciferase expression in mammalian cells. By applying a homogeneous experimental protocol, the specially formulated mixture of substances in this kit significantly extends the time window with steady signal, thereby enabling batch processing of plates in screening assays. This assay kit is optimized for SpectraMax microplate readers from Molecular Devices, with a pre-configured protocol provided for customers using Molecular Devices' SoftMax Pro Software for their data acquisition and analysis.