SpectraMax Glo Steady-Luc Reporter Assay Kit

The SpectraMax® Glo Steady-Luc™ Reporter Assay Kit enables highly sensitive quantitation of firefly luciferase expression in mammalian cells with a long-lived, “glow-type" luminescent signal. The stable luminescent signal is well-suited for multi-plate screening assays using luminescence microplate readers. See Compatible Molecular Devices Microplate Readers on page 6.

Available Kits

Assay Kit	Explorer Kit	Bulk Kit
SpectraMax Glo Steady-Luc Reporter Assay Kit	R8352	R8353

Contents

Product manufactured for Molecular Devices, LLC
1311 Orleans Drive, Sunnyvale, California, United States of America 94089.
Molecular Devices, LLC is ISO 9001 registered.

Chapter 1: About the SpectraMax Glo Steady-Luc Reporter Assay Kit

The SpectraMax Glo Steady-Luc Reporter Assay Kit provides a convenient tool to assay reporter gene activity in cells. The key features of this kit are the following:

- Stable, glow-based luminescent signal, which allows for batch processing of plates in screening assays.
- Optimized for luminescence microplate readers. See Compatible Molecular Devices Microplate Readers on page 6.
- Simplified data acquisition and analysis with preconfigured protocol in the SoftMax® Pro Data Acquisition and Analysis Software.

Assay Principles

Firefly luciferase is a widely used reporter to study gene regulation and function, and for pharmaceutical screening.[1,2] Because of the lack of any endogenous activity in mammalian cells or tissues, it is a very sensitive reporter.[3,4] Firefly luciferase is a 62,000 Dalton protein that is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes ATP-dependent D-luciferin oxidation by oxygen into oxyluciferin (Figure 1-1) with emission of light centered at 560 nm.

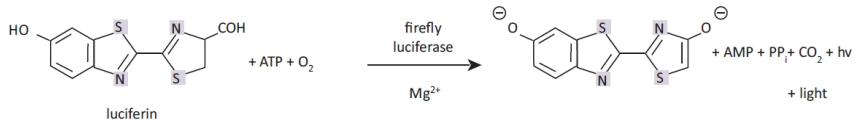

Figure 1-1: SpectraMax Glo Steady-Luc Reporter Catalyzed Reaction For Light Emission

The light that is produced from the reaction leads to formation of suicidal adenyl-oxyluciferin at the enzyme surface, which results in a very short half-life of the light emission with flash-type kinetics. Several substances have been described to prolong light production by regenerating enzyme through removing inhibitory oxyluciferin from the enzyme surface.[5,6]

The SpectraMax Glo Steady-Luc Reporter Assay Kit is a homogeneous, high-sensitivity firefly luciferase reporter gene assay kit for the quantification of firefly luciferase expression in mammalian cells with a signal half-life of about 3 hours (Figure 1-2). It uses a proprietary mixture of substances that modify the enzymatic reaction to produce a longer lasting signal (steady glow) by preventing the formation of adenyl-oxyluciferin at the enzyme surface. Figure 1-2: SpectraMax Glo Steady-Luc Reporter Assay In Transfected CHO-K1 Cells on page 4 illustrates this concept.

Glow-type luciferase assays like the SpectraMax Glo Steady-Luc Reporter assay exchange a somewhat lower luminescence signal compared to flash-type assays for an extension of the assay time window. The sensitivity and limit of detection of the assay depends on the luciferase expression levels in your experimental system as well as the sensitivity of the microplate reader or luminometer. Figure 1-3: Linearity of Purified Luciferase In the SpectraMax Glo Steady-Luc Reporter Assay on page 5 is an example of the concentration response curve of luciferase in this assay on the SpectraMax® i3x Multi-Mode Microplate Reader.

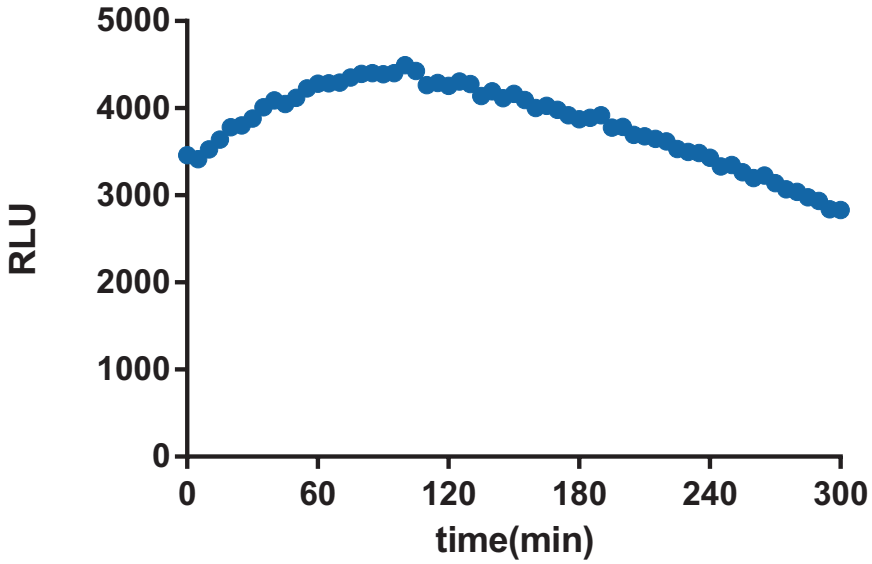

Figure 1-2: SpectraMax Glo Steady-Luc Reporter Assay In Transfected CHO-K1 Cells

CHO-K1 cells were grown in F12-K medium containing 10% FBS in a 6-well plate. Cells were transiently transfected with pGL4 firefly luciferase expression vector (Promega) using Fugene HD transfection reagent (Promega). On the day after transfection, the cells were trypsinized and plated in a white 96-well plate and allowed to grow for 24 hours. On the day of the assay, the plate was equilibrated to room temperature, and then 100 µL reconstituted Steady-Luc working solution containing D-Luciferin was added to each well. The plate was mixed on an orbital shaker for five minutes, and then placed in a SpectraMax i3x Multi-Mode Microplate Reader and mixed. Luminescence was read every five minutes for five hours, with three seconds of orbital shaking before each read.

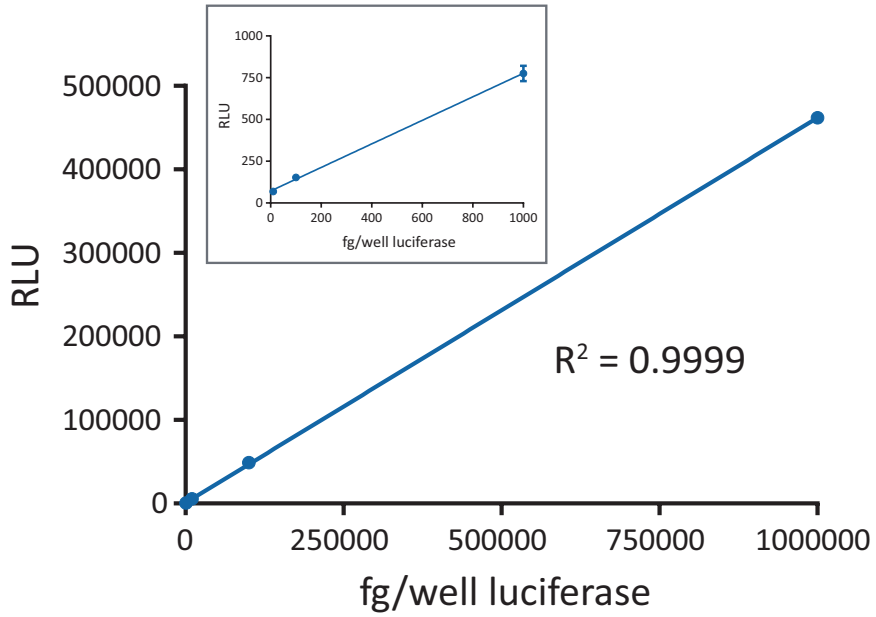

Figure 1-3: Linearity of Purified Luciferase In the SpectraMax Glo Steady-Luc Reporter Assay

Concentrations of purified luciferase ranging from ranging from 1 ng/well to 1 fg/well were pipetted into wells at 100 µL/well. An equal volume of Steady-Luc working solution was added, and the plate was then mixed for 5 minutes on an orbital shaker. The plate was read in luminescence mode on a SpectraMax i3x Multi-Mode Microplate Reader. Luciferase concentrations were chosen to be within a similar RLU range of the cell-based assay shown in Figure 4-1: Cell Titration Experiment With CHO-K1 Cells Transiently Transfected With Firefly Luciferase Vectors on page 9. The inset shows the lower portion of the curve.

Chapter 2: Materials and Equipment

Kit Components

Components of the SpectraMax Glo Steady-Luc Reporter Assay Kit

Item	Explorer Kit (R8352)	Bulk Kit (R8353)
SpectraMax Glo Steady-Luc Assay Buffer (Lyophilized)	1 x 20 mL	1 x 100 mL
Reconstitution Buffer	1 x 20 mL	1 x 100 mL
D-Luciferin	1 x 5 mg	1 x 25 mg

- The Explorer kit is sufficient for two 96-well plates.
- The Bulk kit is sufficient for ten 96-well plates.

The number of plates is based on the example protocols. See Assay Protocols on page 7.

Storage and Handling

Store reagents in this kit at -20°C. When stored as directed, the kit is stable for 6 months from the date it is received.

WARNING! Reagents can contain chemicals that are harmful. Exercise care when handling reagents as described in the related safety data sheet (SDS or MSDS). The safety data sheet is available in the Knowledge Base on the Molecular Devices support web site: support.moleculardevices.com/

Materials Required But Not Provided

Reagents and Supplies

Item	Suggested Vendor
White opaque plates (96-well)	Greiner 655075 or equivalent
White-walled, clear-bottomed assay plates with lid (96-well)	Greiner 655098 or equivalent

Compatible Molecular Devices Microplate Readers

- FilterMax™ F3 Multi-Mode Microplate Reader
- FilterMax™ F5 Multi-Mode Microplate Reader
- FlexStation® 3 Multi-Mode Microplate Reader
- SpectraMax® i3x Multi-Mode Microplate Reader
- SpectraMax® iD3 Multi-Mode Microplate Reader
- SpectraMax® iD5 Multi-Mode Microplate Reader
- SpectraMax® L Microplate Reader
- SpectraMax® M3 Multi-Mode Microplate Reader
- SpectraMax® M4 Multi-Mode Microplate Reader
- SpectraMax® M5 Multi-Mode Microplate Reader
- SpectraMax® M5e Multi-Mode Microplate Reader
- SpectraMax® Mini Multi-Mode Microplate Reader
- SpectraMax® Paradigm® Multi-Mode Microplate Reader

See Microplate Reader Setup With SoftMax Pro Software on page 7.

Chapter 3: Assay Protocols

Considerations Before You Begin

- The example protocol in this document was developed using transiently transfected CHO-K1 cells, and can be adapted for use with other cell types. Assay signals can be optimized by adjusting the cell number and transfection conditions.
- The SpectraMax Glo Steady-Luc Reporter assay should be carried out on cells or samples that are in a culture medium that contains magnesium. The luminescence signal will be reduced in the absence of magnesium.
- The SpectraMax Glo Steady-Luc Reporter luminescence signal has a half-life of approximately three hours, which can fluctuate over time or with temperature variations during the assay. The half-life can also vary depending on the cell culture medium that is used. As a result, raw luminescence values should be compared directly only for those samples that are in the same medium. For comparison of luminescence signal between plates that are read at different times, each plate should include the same common internal control. The luminescence signals from each plate can be normalized to the internal control for the plate.

Microplate Reader Setup With SoftMax Pro Software

The following table displays typical microplate reader settings. In the SoftMax Pro Software, use the preconfigured SpectraMax Quant Steady-Luc Reporter assay protocol in the protocol library.

SpectraMax Glo Steady-Luc Reporter Assay Protocol Settings

Parameter	Setting
Read Mode	Luminescence
Read Type	Endpoint
Wavelengths	All*
PMT and Optics	PMT Gain: Automatic Integration Time: 1 second Read From Top

*Target calibration wavelength for SpectraMax L Microplate Reader: 570 nm

SpectraMax Glo Steady-Luc Reporter Assay Buffer Preparation

To prepare the assay buffer:

1. Allow the kit components to reach room temperature (22°C).
2. Reconstitute the lyophilized SpectraMax Glo Steady-Luc Reporter assay buffer by adding the entire bottle of reconstitution buffer, and then mix by rocking gently until the buffer is a homogeneous solution.

Tip: Reconstitution buffer contains detergent. Mix by rocking gently to avoid excessive foaming.

Note: Reconstituted assay buffer is stable at -20°C for 3 months or -70°C for 6 months. Avoid repeated freeze-thaw cycles.

SpectraMax Glo Steady-Luc Reporter Assay Working Solution Preparation

The volume of SpectraMax Glo Steady-Luc Reporter working solution that is required is equal to the volume of cells and culture medium in each well. For example, if each well contains 100 µL of cells and medium, then 100 µL of working solution per well is required. Prepare only the volume of working solution that is required on the day of the assay.

To prepare the SpectraMax Glo Steady-Luc Reporter working solution:

1. Mix the D-luciferin substrate and the SpectraMax Glo Steady-Luc Reporter assay buffer in a 1 mg to 4 mL ratio:
   - For each lyophilized 5 mg vial of D-luciferin, mix with 20 mL of reconstituted assay buffer.
   - For each lyophilized 25 mg vial of D-luciferin, mix with 100 mL of reconstituted assay buffer.
2. Mix by inversion until the substrate is completely dissolved.

Note: D-luciferin is limited in stability when diluted. If you do not plan to use a full vial on the same day, then dilute the vial to 10 mg/mL in water and store aliquots at -20°C. D-luciferin is stable for one month when diluted and stored under these conditions. You can prepare the required volume of Steady-Luc Reporter working solution by diluting D-luciferin in reconstituted assay buffer to a final concentration of 0.25 mg/mL (2.5 µL of 10 mg/mL D-luciferin stock solution per 100 µL assay buffer).

Assay Setup

The following was used for an endpoint luminescence assay with luciferase in a 96-well format. Optimal assay conditions for different cell types and plate formats can vary. You should test a range of parameters to determine the optimal conditions for your assay system.

To set up the assay:

1. Remove the plates that contain luciferase-expressing cells from the incubator and allow them to come to room temperature.
2. Add a volume of SpectraMax Glo Steady-Luc Reporter working solution that is equal to the volume of the cells and culture medium in each well. For example, for 96-well plates, add 100 µL of working solution to each well that contains 100 µL of cells in medium, for a final volume of 200 µL per well.
3. Mix on an orbital shaker for at least 5 minutes to ensure complete lysis of cells.
4. Immediately before measuring the luminescence signal, make sure to mix the samples thoroughly again, and then measure the signal with a microplate reader that has luminescence detection capability or with a luminometer. See Microplate Reader Setup With SoftMax Pro Software on page 7.

Note: In the SoftMax Pro Software, use the SpectraMax Glo Steady-Luc Reporter assay protocol in the protocol library or on the protocol sharing web site (www.softmaxpro.org).

Chapter 4: Data Analysis Examples

Example Results

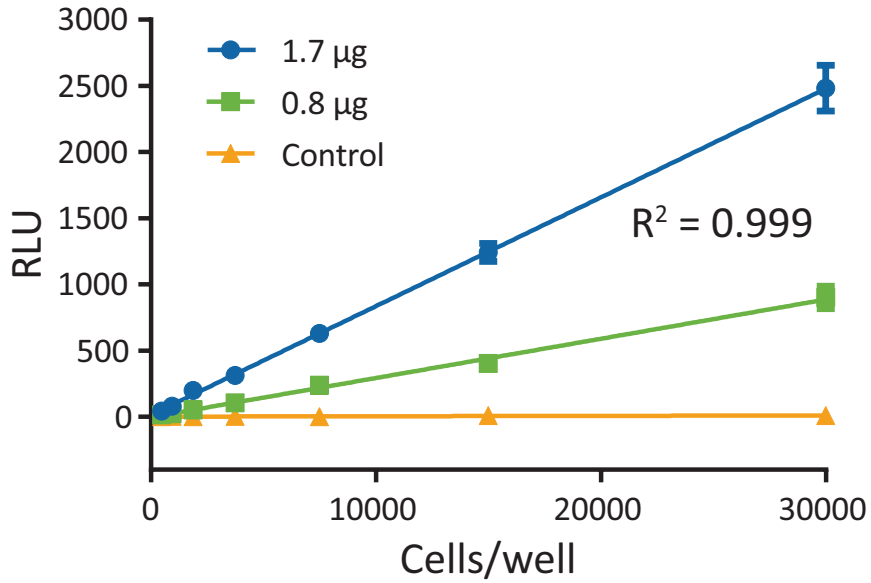

Figure 4-1: Cell Titration Experiment With CHO-K1 Cells Transiently Transfected With Firefly Luciferase Vectors

CHO-K1 cells were seeded in 6-well plates at 250,000 cells per well and grown in Ham's F-12 medium + 10% FBS overnight. CHO-K1 cells were transiently transfected with 1.7 µg or 0.8 µg per well of pGL4.13[luc2/SV40] vector, which encodes the luciferase gene *luc*2 under control of the SV40 early enhancer/promoter, or pGL3-Basic (control) vector, which lacks promoter and enhancer sequences. After 24 hours, cells were trypsinized and seeded at 100 µL/well into a 96-well plate starting at 30,000 cells/well with subsequent two-fold dilutions. 100 µL/well Steady-Luc Reporter working solution was added to the wells. The plate was mixed using an orbital shaker. After 10 minutes, luminescence was measured and recorded on the SpectraMax i3x Multi-Mode Microplate Reader using the preconfigured SpectraMax Glo Steady-Luc Reporter assay protocol that is available in the SoftMax Pro Software protocol library in the software or on the protocol sharing web site (www.softmaxpro.org). The assay shows a linear relationship between luminescence and cell number.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website—support.moleculardevices.com/—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Please have the instrument serial number (on the rear of the instrument), and any related sample data files available when you call.

References

1. Alam, J. and J.L. Cook. 1990. *Anal. Biochem.* **188**:245-254.

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