

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 such as the SpectraMax® microplate readers with luminescence detection capability.

Table 1-1: Available Kits

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

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SpectraMax Glo Steady-Luc Reporter Assay Kit

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

The SpectraMax Glo Steady-Luc Reporter Assay Kit provides a convenient tool for assaying 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, such as SpectraMax readers.
- Simplified data acquisition and analysis with preconfigured protocol in SoftMax® Pro Software.

Assay Principles

Firefly luciferase is a widely used reporter for studying 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.

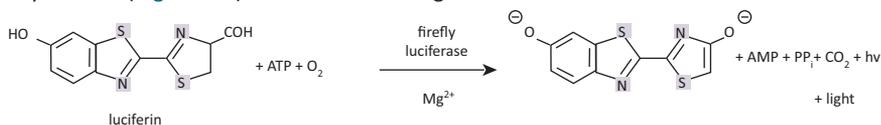


Figure 1-1: SpectraMax Glo Steady-Luc Reporter catalyzed reaction for light emission

The light that is produced from the reaction leads to formation of suicidal adenylyl-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 adenylyl-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 chosen plate 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.

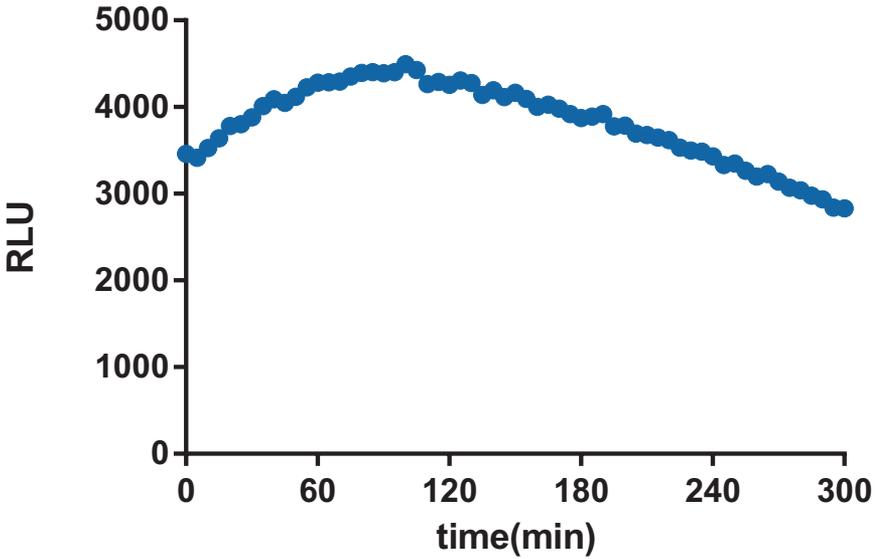


Figure 1-2: SpectraMax Glo Steady-Luc Reporter Assay in transfected CHO-K1 cells

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 μ L 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.

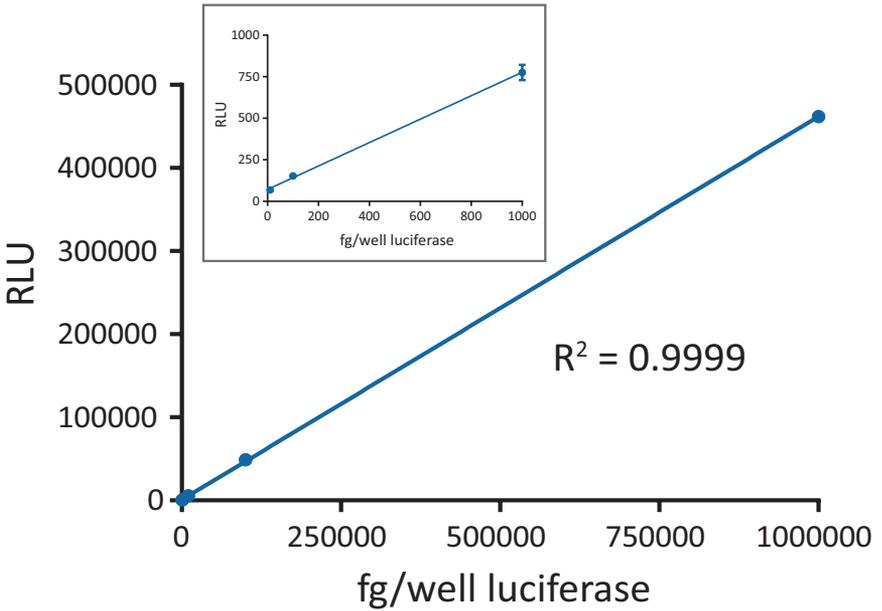


Figure 1-3: Linearity of purified luciferase in the SpectraMax Glo Steady-Luc Reporter assay

Concentrations of purified luciferase ranging from ranging from 1 ng/well to 1 fg/well were pipetted into wells at 100 μ l/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

Table 2-1: 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 microplates.
- The Bulk kit is sufficient for ten 96-well microplates.

The number of microplates is based on the example protocol that is detailed in this document.

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: www.moleculardevices.com/support

Materials Required but Not Provided

Table 2-2: Reagents and Supplies

Item	Suggested Vendor
White opaque microplates (96-well)	Greiner 650076 or equivalent
White-walled, clear-bottomed assay plates (96-well)	Greiner 655088 or equivalent

Compatible Molecular Devices Microplate Readers

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

For information about setting up your instrument, see [Microplate Reader Setup with SoftMax Pro Software on page 7](#).

Chapter 3: Assay Protocols

Considerations Before you Begin

- The example protocol that is detailed 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

Table 3-1 displays typical microplate reader settings. In SoftMax® Pro Software, use the preconfigured SpectraMax Quant Steady-Luc Reporter assay protocol that is available in the protocol library.

Table 3-1: 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

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. Mixing by rocking gently avoids 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 required volume of SpectraMax Glo Steady-Luc Reporter working solution, do the following:

- 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.
- 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. Molecular Devices recommends testing a range of parameters to determine the optimal conditions for your assay system.

Use the following procedure to set up the assay:

- Remove the plates that contain luciferase-expressing cells from the incubator and allow them to come to room temperature.
- 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.
- Mix on an orbital shaker for at least 5 minutes to ensure complete lysis of cells.
- Immediately before measuring the luminescence signal, make sure to mix the samples thoroughly again, and then measure the signal with a plate reader that has luminescence detection capability or with a luminometer. See [Microplate Reader Setup with SoftMax Pro Software on page 7](#).



Note: In SoftMax[®] Pro Software, use the preconfigured SpectraMax Glo Steady-Luc Reporter assay protocol that is available in the protocol library in the software or on the protocol sharing web site (www.softmaxpro.org).

Chapter 4: Data Analysis Examples

Example Results

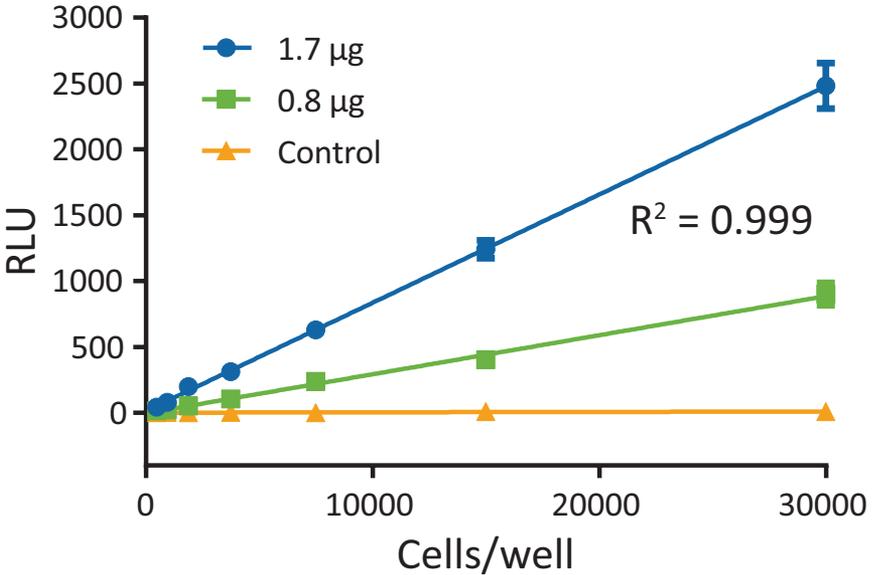


Figure 4-1: Cell titration experiment with CHO-K1 cells transiently transfected with firefly luciferase vectors

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 *luc2* 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.

Chapter 5: 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 possible level of technical service.

Our support web site, www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

Please have the product name, part number, and lot number available when you call.

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