SpectraMax DuoLuc Reporter Assay Kit

The SpectraMax® DuoLuc™ Reporter Assay Kit enables highly sensitive quantitation of both firefly and *Renilla* luciferases in mammalian cells. Serial injection of two optimized detection reagents allows the luciferases to be assayed in the same microplate well. The assay is well-suited for microplate readers that are equipped with injectors such as the SpectraMax® iD3 Multi-Mode Microplate Reader that includes the SpectraMax® Injector System with SmartInject™ Technology.

Table 1-1: Available Kits

Assay Kit	Evaluation Kit	Explorer Kit	Bulk Kit
SpectraMax DuoLuc Reporter Assay Kit	R8360	R8361	R8362

Contents

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Chapter 1: Materials and Equipment

Kit Components

Table 1-1: Components of the SpectraMax DuoLuc Reporter Assay Kit

Item	Evaluation Kit 50 Reactions	Explorer Kit 200 Reactions	Bulk Kit 1000 Reactions
Passive Lysis Buffer, 1X	1 x 5 mL	1 x 20 mL	1 x 100 mL
Firefly Assay Buffer	1 x 6 mL	1 x 25 mL	1 x 110 mL
Firefly Substrate	1 x 1.1 mg	2 x 2.2 mg	10 x 2.2 mg
Renilla Assay Buffer	1 x 6 mL	1 x 25 mL	1 x 110 mL
Aquaphile™ Coelenterazine	1 x 220 µg	2 x 440 µg	10 x 440 µg

Sufficient reagents are included to run the number of assays indicated for each kit using the example setup method in this document. Ample Passive Lysis Buffer is provided to run the indicated number of assays with cells that are grown in a microplate format. For cells grown in flasks or other vessels that may require more lysis buffer, additional Passive Lysis Buffer (P/N R8363) can be purchased separately.

Storage and Handling

Store the kit at -80°C. Passive Lysis Buffer, Firefly Substrate, and Aquaphile Coelenterazine may be stored at -20°C, if desired. When stored as directed, the kit is stable for 3 months from the date it is received. All kit components are stable for up to 5 freeze/thaw cycles.

WARNING! Reagents can contain chemicals that are harmful. Exercise care when handling reagents as described in the related safety data sheet (SDS). 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

Solid white microplates or white-walled, clear-bottomed microplates (if cells are to be cultured and viewed in the same plate in which they are assayed)

Deionized water (dH_20)

Phosphate-buffered saline (PBS)

Rocking platform or orbital shaker

Luminescence microplate reader equipped with dual injectors

Compatible Molecular Devices Microplate Readers

- SpectraMax iD3 Multi-Mode Microplate Reader that includes the SpectraMax Injector System with SmartInject Technology
- SpectraMax® i3x Multi-Mode Microplate Reader with SpectraMax® Injector Cartridge
- SpectraMax® L Microplate Reader

Use the preconfigured protocols included in SoftMax® Pro Software to perform reads.

Chapter 2: About the SpectraMax DuoLuc Reporter Assay Kit

The SpectraMax DuoLuc Reporter Assay Kit enables the measurement of both firefly and *Renilla* luciferase activity in cells. Key features of the kit are as follows:

- Dual reagents allow detection of both firefly and *Renilla* luciferase activities in the same microplate well.
- Assay is optimized for microplate readers equipped with injectors.
- Data acquisition and analysis are simplified with a preconfigured protocol in SoftMax Pro Software.

Assay Principles

Firefly luciferase is a widely used reporter to study gene regulation and function.[1,2] It is a very sensitive reporter due to the lack of any endogenous luciferase activity in mammalian cells or tissue.[3,4] Firefly luciferase is a 62-kDa protein that is active as a monomer and does not require subsequent processing for its activity. It catalyzes ATP-dependent oxidation of D-luciferin with the resulting emission of light.

Luciferase from the sea pansy *Renilla reniformis* is often used in multiplexed luciferase assays as a second reporter for normalizing transfection efficiency and for studying gene regulation and function.[5,6,7] *Renilla* luciferase catalyzes coelenterazine oxidation by oxygen to produce light.[8]

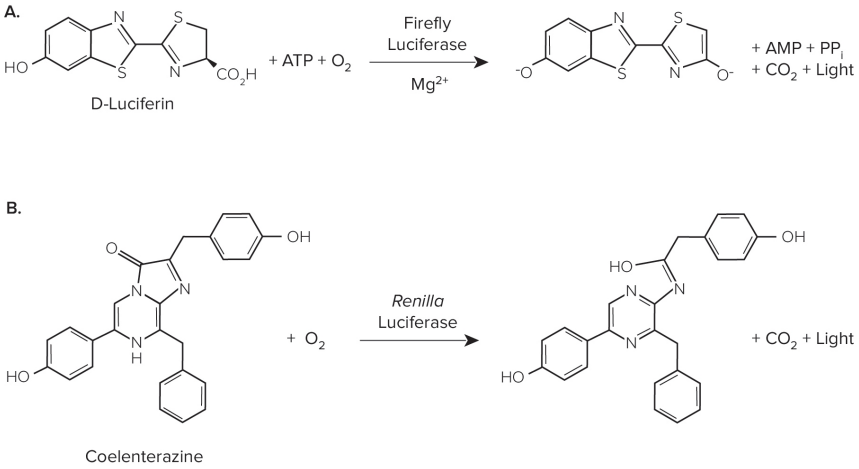

Figure 2-1: Bioluminescent reactions catalyzed by (A) firefly luciferase and (B) *Renilla* luciferase

Because firefly and *Renilla* luciferases have different enzyme structure and substrate requirements, their bioluminescent reactions can be monitored sequentially in the same assay well. First a working solution containing D-luciferin is added to the sample, and firefly luciferase luminescence is measured. A second solution containing coelenterazine and a firefly quencher is then added, and *Renilla* luciferase luminescence is measured.

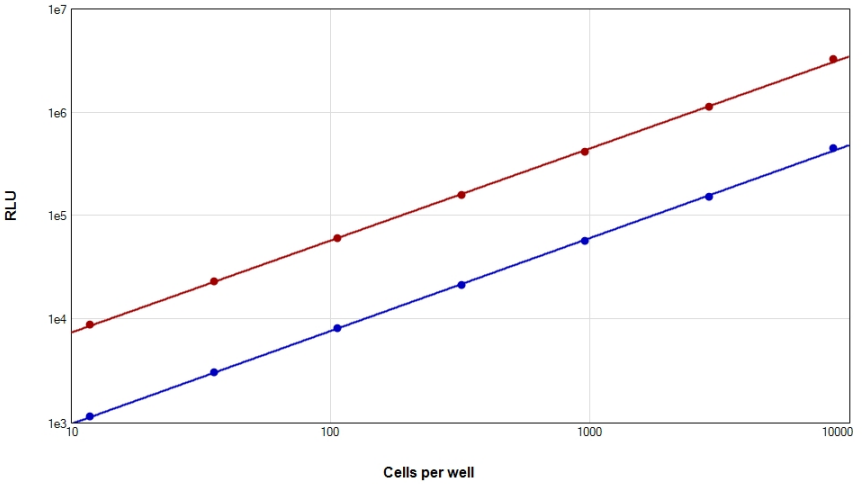

Figure 2-2: Cell based luciferase assay example

The SpectraMax DuoLuc Reporter Assay Kit was used to measure luciferase activity in lysates from HeLa cells that were co-transfected with firefly (red plot) and *Renilla* (blue plot) luciferases. Luminescence was measured on a SpectraMax i3x Multi-Mode Microplate Reader with Injector Cartridge, using a preconfigured protocol in SoftMax Pro Software.

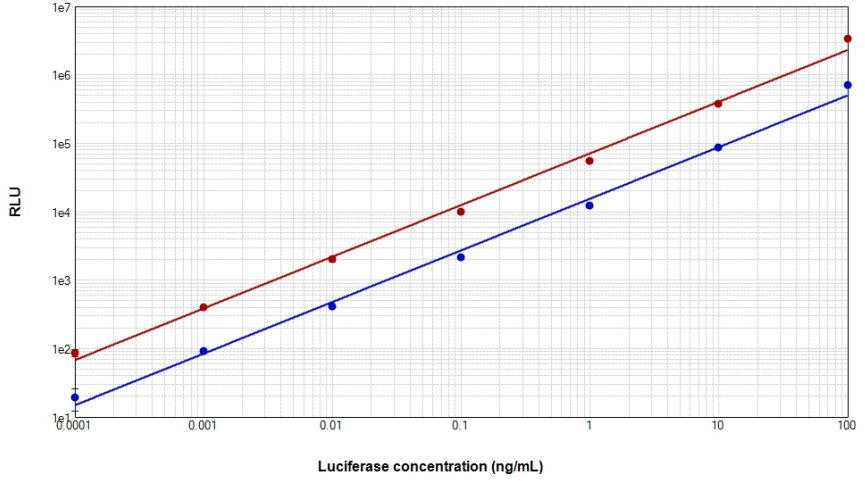

Figure 2-3: Firefly and *Renilla* luciferase standard curves

Purified luciferases with concentrations spanning six orders of magnitude were assayed using the SpectraMax DuoLuc Reporter Assay Kit, and the resulting RLU values with firefly (red plot) and *Renilla* (blue plot) were plotted using a log-log fit (r^2= 0.99).

Chapter 3: Assay Protocols

Considerations Before You Begin

- **Cell Preparation:** The example protocol that is detailed in this document was developed using transiently transfected HeLa cells, but it can be adapted for use with other cell types. Transfection conditions will need to be optimized for the cells, luciferase vectors, and transfection reagent that will be used.
- **Assay Background Subtraction:** Non-transfected cells, or passive lysis buffer only, may be used as a control for background luminescence of the assay. Luminescence of the control is subtracted from that of the transfected cell samples to obtain a more accurate measurement of luciferase activity.

Preparation of Cell Lysates

The following method was designed for use with cells assayed in a 96-well microplate format.

1. Allow Passive Lysis Buffer to reach room temperature (22°C).

   Note: Passive Lysis Buffer is stable for up to five freeze-thaw cycles. Aliquotting may be desired for ease of handling.

2. Remove growth medium from the cultured cells, and then gently wash the cells once with 100 µL phosphate buffered saline (PBS) per well.
3. Remove PBS and add 20 µL of Passive Lysis Buffer to each well. For other plate formats refer to Table 3-1

   Table 3-1: Passive Lysis Buffer Volumes for Different Plate Formats

Plate Format	Passive Lysis Buffer/Well
6-well	500 µL
12-well	250 µL
24-well	100 µL
48-well	65 µL
96-well	20 µL

4. To ensure lysis, place the culture plates on a rocking platform or orbital shaker and gently rock/shake the culture plates at room temperature for 15 minutes.
5. Prepare lysates for assay in one of the following ways:
   - If the cells were grown and lysed in a 96-well microplate with white walls, or a solid white microplate, then lysates can be assayed directly in the microplate. If the assay is to be done on the same day, the microplate can be sealed and stored at 4°C. Otherwise it can be stored at -20°C or -80°C.
   - If the cells were grown in a format other than a 96-well microplate, transfer 20 µL of lysate to each well of a solid white 96-well microplate for assay. Microplates containing lysate may be stored as indicated above.

Firefly Working Solution Preparation

Prepare enough firefly working solution to do the anticipated number of reactions, with 100 µL of working solution required per reaction. Additional volume is required to prime the injector. For optimal results, prepare the working solution fresh before each use and use it within three hours of preparation. Firefly working solution activity decreases by approximately 10% after three hours, and by approximately 25% after five hours at room temperature.

To prepare the required volume of firefly working solution, do the following:

1. Thaw Firefly Assay Buffer at room temperature.
2. Prepare a 10 mg/mL Firefly Substrate (D-luciferin) stock solution as follows:
   - For the Evaluation Kit (1.1 mg), add 110 µL of dH_20 to the vial and mix.
   - For the Explorer or Bulk Kit (2.2 mg), add 220 µL of dH_20 to a single vial and mix.

   Note: The Firefly Substrate stock solution can be stored at -20°C or below for up to 6 months, and is stable up to 5 freeze/thaw cycles.
3. Dilute the Firefly Substrate stock solution in Firefly Assay Buffer at a ratio of 1:50. For example, add 220 µL of Firefly Substrate stock solution to 11 mL of Firefly Assay Buffer.

Renilla Working Solution Preparation

Prepare enough *Renilla* working solution to do the anticipated number of reactions, with 100 µL of working solution required per reaction. Additional volume is required to prime the injector. For optimal results, prepare the working solution fresh before each use and use the working solution within three hours of preparation. *Renilla* background increases up to 60% after five hours at room temperature. Prepare the required volume of *Renilla* working solution as follows:

1. Thaw *Renilla* Assay Buffer at room temperature.
2. Prepare the 2 mg/mL Aquaphile Coelenterazine stock solution as follows:
   - For the Evaluation Kit (220 µg), add 110 µL of dH_2O to the vial and mix.
   - For the Explorer Kit or Bulk Kit (440 µg), add 220 µL of dH_20 to a single vial and mix.

   Note: Aquaphile Coelenterazine stock solution can be stored at -20°C or below for up to 3 months, and is stable up to 5 freeze/thaw cycles.
3. Dilute the 2 mg/mL Aquaphile Coelenterazine stock solution in *Renilla* Assay Buffer at a ratio of 1:50. For example, add 220 µL of Aquaphile Coelenterazine stock solution to 11 mL of *Renilla* Assay Buffer.

Assay Setup

When you use a SpectraMax microplate reader with SoftMax Pro Software, preconfigured protocols are available in the software's Protocol Library. The protocols include optimized settings and analysis of results.

To perform the SpectraMax DuoLuc assay in a 96-well microplate format using a luminescence microplate reader with injectors, follow the steps bellow:

Note: Configure your microplate reader to perform the following steps for each assay well. All steps are completed on one well before continuing to the next well.

1. Use injector 1 to add 100 µL of firefly working solution to the well.
2. Delay for 2 seconds to allow the reaction to develop.
3. Measure luminescence with an integration time of 5 seconds.
4. Use injector 2 to add 100 µL of *Renilla* working solution to the well.
5. Delay for 2 seconds to allow the reaction to develop.
6. Measure luminescence with an integration time of 5 seconds.

For each assay well, normalize the RLU value of the first measurement (firefly luciferase) to that of the second (*Renilla* luciferase).

Obtain 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 seek, follow the links to the Technical Support Service Request Form to send an email to our technical support representatives.

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

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