

# SpectraMax DuoLuc Reporter Assay Kit

Dual luciferase measurement for your microplate reader

### **KEY FEATURES**

- Flash-type luminescence with excellent sensitivity for firefly and *Renilla* luciferase detection
- Expression measurement in as few as ten cells per well
- Support of your complete solution of reader, software and assay

Reporter gene assays are an important tool for researchers to monitor cellular events associated with gene expression, gene regulation, and signal transduction. A reporter gene such as firefly luciferase is typically inserted adjacent to a gene or promoter of interest, and its expression increases or decreases concomitantly. Luciferase from the sea pansy Renilla reniformis is often used in multiplexed luciferase assays, where it serves as a second reporter against which the firefly signal is normalized. Under the control of a constitutive promoter, Renilla luciferase signal provides a readout for cell number and transfection efficiency.

Firefly luciferase, one of the most commonly used reporters, produces light by catalyzing a bioluminescence reaction that oxidizes D-luciferin in the presence of oxygen and ATP. *Renilla* luciferase catalyzes coelenterazine oxidation to produce light. Due to the difference in substrate requirements, it is possible to measure the activity of both luciferases in a single sample through sequential addition of the substrates. The result is a convenient assay with two readouts that can be normalized to each other to provide more complete information about a cellular pathway or event.

### Optimized assay for Molecular Devices readers

The SpectraMax<sup>®</sup> DuoLuc<sup>™</sup> Reporter Assay Kit enables highly sensitive quantitation of both firefly and Renilla luciferases in mammalian cells. Serial injection of two optimized detection reagents allows both luciferases to be assayed in the same microplate well. Addition of the firefly substrate and measurement of the resulting light is followed by addition of Renilla substrate plus a quencher for the firefly luciferase reaction, followed by a second light measurement. The assay is well-suited for microplate readers equipped with dual injectors that can deliver each reagent sequentially. The assay kit was optimized on the Molecular Devices SpectraMax® i3x Multi-Mode Microplate Reader with Injector Cartridge using a preconfigured protocol provided in SoftMax® Pro Software to simplify data acquisition and analysis (Figure 1).



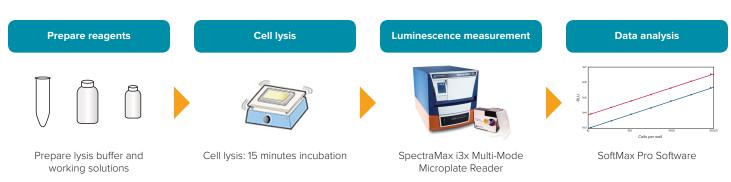
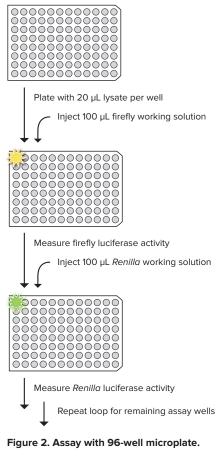


Figure 1. Representative experimental workflow for dual luciferase assay.

## Optimized assay for microplate format

The DuoLuc assay workflow is performed automatically in SpectraMax microplate readers equipped with dual injectors, using a preconfigured protocol in SoftMax Pro Software. Each sample is processed in turn for consistent well-to-well data (Figure 2).



#### Contact Us

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 Check our website for a current listing of worldwide distributors.

### Optimal data quality for dual reporter assay

The DuoLuc kit offers a broad dynamic range and optimal sensitivity using microplate readers equipped with injectors. A linear detection range ( $r^2 > 0.99$ ) spanning at least six decades is achieved with purified luciferase enzymes (Figure 3). The assay can detect luciferase activity in fewer than 12 cells per well (Figure 4). Sensitivity and a broad linear detection range offer highly accurate quantitation of reporter gene activity under a wide variety of cell culture and experimental conditions.

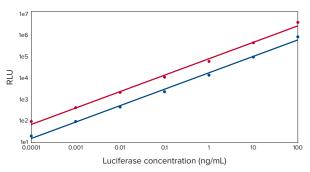


Figure 3. Dilution series of purified firefly (red plot) and *Renilla* (blue plot) luciferases. Measured using the SpectraMax DuoLuc Reporter Assay Kit on the SpectraMax i3x microplate reader.

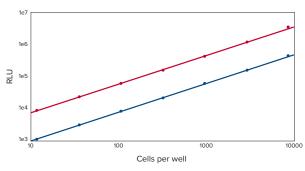


Figure 4. Dilution series of HeLa cells transfected with firefly (red plot) and *Renilla* (blue plot) **luciferases.** Measured using the SpectraMax DuoLuc Reporter Assay Kit on the SpectraMax i3x microplate reader.

| Ordering information                  | Part number             |                          |                           |
|---------------------------------------|-------------------------|--------------------------|---------------------------|
|                                       | Evaluation              | Explorer                 | Bulk                      |
| SpectraMax DuoLuc Reporter Assay Kit* | R8360<br>(50 reactions) | R8361<br>(200 reactions) | R8362<br>(1000 reactions) |

\*Compatible with Molecular Devices microplate readers

SpectraMax<sup>®</sup> iD3 Multi-Mode Microplate Reader
SpectraMax<sup>®</sup> i3/i3x Multi-Mode Microplate Reader

SpectraMax<sup>®</sup> L Microplate Reader

Additional Passive Lysis Buffer R8363 is also available.

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