

Renilla-Firefly Luciferase Dual Assay Kit

1 Contents

Components	HY-K1013-100T
Firefly Luciferase Buffer	10 mL
Firefly Luciferase Substrate (Lyophilized)	1 vial
Renilla Luciferase Buffer	10 mL
Renilla Luciferase Substrate (125×)	80 µL
Lysis Buffer (5×)	10 mL

2 General Information

MCE Renilla-Firefly Luciferase Dual Assay Kit is designed to be used for high-throughput, rapid quantitation of both Firefly and Renilla luciferases from a single sample in mammalian cell culture. Firefly luciferase produces a bioluminescent signal ($\lambda_{\max}=570$ nm) that results from the oxidation of D-Luciferin in the presence of ATP and Mg^{2+} and O_2 (Figure 1). While Renilla luciferase produces a bioluminescent signal ($\lambda_{\max}=480$ nm) results from the oxidation of coelenterazine (Figure 2). Renilla luciferase is commonly used as a normalizing transfection control for Firefly reporter assays.

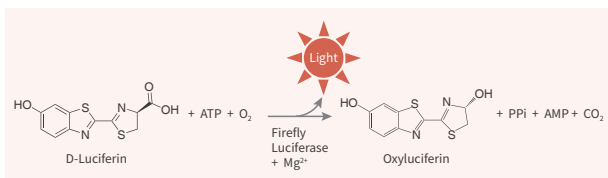


Figure 1. Chemical reaction of D-Luciferin and Firefly Luciferase.

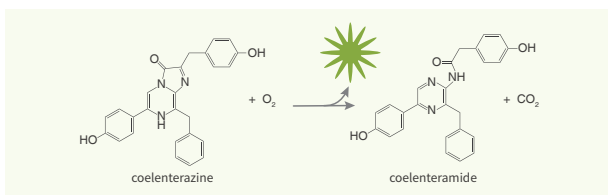


Figure 2. Chemical reaction of coelenterazine and Renilla Luciferase.

MCE Renilla-Firefly Luciferase Dual Assay Kit contains high-purity D-Luciferin, coelenterazine and proprietary reaction buffer. The Firefly Luciferase Working Solution is first added to the sample. This reagent contains D-Luciferin which can produce Firefly luciferase bioluminescence. Next, the Renilla Luciferase Working Solution is added to the same sample. It quenches the Firefly luciferase bioluminescence and produce Renilla luciferase bioluminescence. The light production of both reactions can be conveniently measured on a luminometer.

3 General Protocol

1. Material Preparation

1.1 Preparation of Lysis Buffer (1×)

Dilute Lysis Buffer (5×) 1:5 with dH_2O (e.g. 1 mL Lysis Buffer + 4 mL dH_2O)

1.2 Preparation of Firefly Luciferase Working Solution

Briefly centrifuge tube of Firefly Luciferase Substrate. Prepare the Firefly Luciferase Working Solution by resuspending the lyophilized Firefly Luciferase Substrate in 10 mL Firefly Luciferase Buffer.

1.3 Preparation of Renilla Luciferase Working Solution

Briefly centrifuge tube of Renilla Luciferase Substrate (125×). Prepare the Renilla Luciferase Working Solution by adding 80 µL Renilla Luciferase Substrate (125×) to 10 mL Renilla Luciferase Buffer. Mix well by inverting the tube several times.

Note: Firefly Luciferase Working Solution and Renilla Luciferase Working Solution can be dispensed into aliquots. When protecting from light and avoiding repetitive freeze-thaw cycles, the reagent is stable for one month at $-20^{\circ}C$ or for one year when stored at $-80^{\circ}C$.

2. Cell Lysis

2.1 Determine transfection parameters (plated cell density and subsequent incubation time) to ensure that cells are no more than 95% confluent at the desired time of lysate preparation.

2.2 Carefully remove culture medium from cells. Wash cells with wash solution, such as PBS, normal saline or serum-free culture medium, to remove residual medium.

2.3 Add proper volume of Lysis Buffer (1×). The recommended volumes of Lysis Buffer (1×) to add per well are as follows:

Multi-well Plate	Lysis Buffer/Well
6-well	500 μ L
12-well	200 μ L
24-well	100 μ L
48-well	50 μ L
96-well	30 μ L

Note: The volume of added Lysis Buffer (1 \times) can be adjusted proportionally.

2.4 Place the culture plates on a shaker with gentle shaking at room temperature for 15 minutes.

2.5 After lysis, centrifuge at 12,000 rpm for 10 minutes at 4 °C. Transfer the supernatants to a new tube for further analysis.

3. Luciferase Dual Assay

3.1 Add 20 μ L of cell lysate to a black flat-bottomed 96-well plate.

3.2 Add 100 μ L of Firefly Luciferase Working Solution into each well containing cell lysate.

3.3 Immediately after adding the reagent, read the sample to capture the Firefly luciferase signals.

3.4 Add 100 μ L of Renilla Luciferase Working Solution into each well from step 3.3, then immediately read the sample again to capture the Renilla luciferase signals.

3.5 Calculate the ratio of luminescence from the Firefly luciferase to the Renilla luciferase.

Note: 1) Assure Firefly Luciferase Working Solution and Renilla Luciferase Working Solution are equilibrated to room temperature before use.

2) The luminescence values of Firefly and Renilla Luciferase should be subtracted from the corresponding background values.

4 Storage

Stored at -20°C protecting from light, and is stable for up to 12 months

5 Precautions

1. Assure the reagent is equilibrated to room temperature before use.

2. Because the luminescent signals are affected by assay conditions, raw results should be compared only between samples measured at the same time and using the same medium/serum combination.

3. Firefly Luciferase Working Solution and Renilla Luciferase Working Solution are stable for 1 month at -20°C or for 1 year when stored at -80°C. Protect from light and avoid repetitive freeze-thaw cycles.

4. This product is for R&D use only, not for drug, house-hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.