LifeSct.com
Life Scientists' Service Center

Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
14323 Woodcrest Dr., Rockville, MD 20853

SuperLumin™ Dual Luciferase Assay Kit

Cat. #: M0063 Size: 100 T

Introduction

SuperLumin™ Dual Luciferase Assay Kit provides a simple and robust assay system for measuring Firefly and Renilla luciferase activities from a single sample, and is designed to allow high-throughput analysis of mammalian cells containing genes for firefly and Renilla luciferases grown in 96- or 384-well plates (Figure 1). The SuperLumin™ Dual Firefly Luciferase Reagent can be added directly to cells in growth medium without washing or preconditioning. This reagent induces cell lysis and acts as a substrate for firefly luciferase, which has a half-life of approximately 1 hour. Addition of the SuperLumin™ Dual Renilla Luciferase Reagent quenches the luminescence from the firefly reaction by at least 10,000-fold and provides the substrate for Renilla luciferase in a reaction that can also be read within 2 hours. The kit is designed to work in growth media commonly used for mammalian cells with or without added serum.

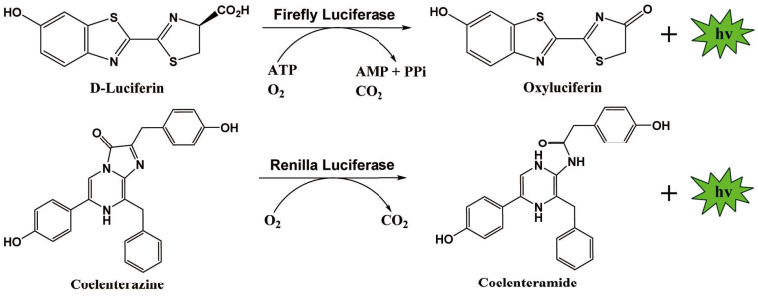

Figure 1. Bioluminescent reactions catalyzed by Firefly and Renilla luciferases

Package Information

Components	M0063
Dual Firefly Luciferase Substrate	100 µl
Dual Firefly Luciferase Buffer	10 ml
Dual Renilla Luciferase Substrate	100 µl
Dual Renilla Luciferase Buffer	10 ml

Storage

Store at -20°C and protect from light.

Preparation before the experiment and precautions

1. Culture cells in multi-well plates which are compatible with the type of luminometer for cell culture.

2. The luciferase assay is temperature sensitive, make sure the reagents be equilibrated to room temperature before measurement.

3. The luminescence signal is affected by assay conditions, results should be compared only between samples measured using the same media/serum combinations and at the same time.

4. To achieve linear assay performance at low light levels, the background luminescence must be subtracted from all readings.

Protocol

1. Place the frozen Dual Firefly Luciferase Substrate, Dual Firefly Luciferase Buffer, Dual Renilla Luciferase Substrate and Dual Renilla Luciferase Buffer in a water bath at room temperature. Mix well after thawing.

2. Prepare the Dual Firefly Luciferase Assay Reagent by diluting Dual Firefly Luciferase Substrate at 1:100 with Dual Firefly Luciferase Buffer. Mix well by inverting the tube several times.

For example: If you are running 100 tests, add 100 µl of Dual Firefly Luciferase Substrate to 10 ml of Dual Firefly Luciferase Buffer.

3. Prepare the Dual Renilla Luciferase Assay Reagent by diluting Dual Renilla Luciferase Substrate at 1:100 with Dual Renilla Luciferase Buffer. Mix well by inverting the tube several times.

For example: If you are running 100 tests, add 100 µl of Dual Renilla Luciferase Substrate to 10 ml of Dual Renilla Luciferase Buffer.

4. Remove multiwell plates containing mammalian cells from the incubator, and equilibrate cultured cells to room temperature. The plates must be compatible with luminescence measurement in the luminometer being used.

5. Add a volume of Dual Firefly Luciferase Assay Reagent equal to that of culture medium in each well and mix. For 96-well plates, typically 75 µl of reagent is added to cells grown in 75 µl of medium. For 384-well plates, typically 20 µl of reagent is added to cells grown in 20 µl of medium.

6. Place the culture plates on a rocking platform or orbital shaker with gentle rocking/shaking for ~10 min.

7. Measure the firefly luminescence in a luminometer (consult the instrument manual). Optimal results will be generated if the luminescence is measured within 1 hours of addition of Dual Firefly Luciferase Assay Reagent.

8. Add a volume of Dual Renilla Luciferase Assay Reagent equal to that of culture medium in each well and mix. As noted in Step 5, this volume is typically 75 µl for 96-well plates and 20 µl for 384-well plates.

9. Wait for 5 min, then measure luminescence. Renilla luminescence should be measured in the same plate order as the firefly luminescence was measured (Step 7). Optimal results will be generated if the luminescence is measured within 1 hours of addition of Dual Renilla Luciferase Assay Reagent.

10. Calculate the ratio of luminescence from the experimental reporter to luminescence from the control reporter.