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ABP Dual Luciferase Assay Kit

Catalog Number: FP308, FP309

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
ABP Dual Luciferase Assay Kit (Cat. No. FP308)				The product is stable for at least 6 months when stored as directed.
Dual Firefly Luciferase Substrate (Component A)	100 µL	100X	-20 °C Protect from light	
Dual Firefly Luciferase Buffer (Component B)	10 mL	1X		
Dual Renilla Luciferase Substrate (Component C)	100 µL	100X		
Dual Renilla Luciferase Buffer (Component D)	10 mL	1X		
ABP Dual Luciferase Assay Kit (Cat. No. FP309)				
Dual Firefly Luciferase Substrate (Component A)	500 µL	100X	-20 °C Protect from light	
Dual Firefly Luciferase Buffer (Component B)	50 mL	1X		
Dual Renilla Luciferase Substrate (Component C)	500 µL	100X		
Dual Renilla Luciferase Buffer (Component D)	50 mL	1X		

Product Description

Transcriptional regulation using a reporter gene expression is routinely used to study a wide range of physiological events in the biotechnology and pharmaceutical industries. Traditionally, the ease and sensitivity of firefly luciferase assays have made it relatively simple to monitor the upregulation of genetic elements. However, it has been more difficult to measure downregulation of genes because of the difficulty in discriminating between cell death and cellular downregulation. Normalizing the expression of an experimental reporter to the expression of a control reporter can help differentiate between specific and nonspecific cellular responses.

Firefly and *Renilla* luciferases are widely used as co-reporters for these normalized studies because both assays are quick, easy and sensitive. Firefly luciferase is a 61 kDa and *Renilla* luciferase a 36kDa protein. Both are monomeric and neither requires post-translational processing, so they can function as genetic reporters immediately upon translation.

The ABP 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 ABP 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 ABP 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 ABP Dual Luciferase Assay Kit is designed to work in growth media commonly used for mammalian cells with or without added serum with following features:

- Simplicity: Directly lyse cells in culture medium and measure luciferase activities without washing.
- Signal stability: The luminescent signal is highly stable with signal half-life of ~1 hr.
- Versatility: The assay is compatible with many eukaryotic cells (adherent or suspended).
- High-throughput: The assay system is adopted to high-throughput application.

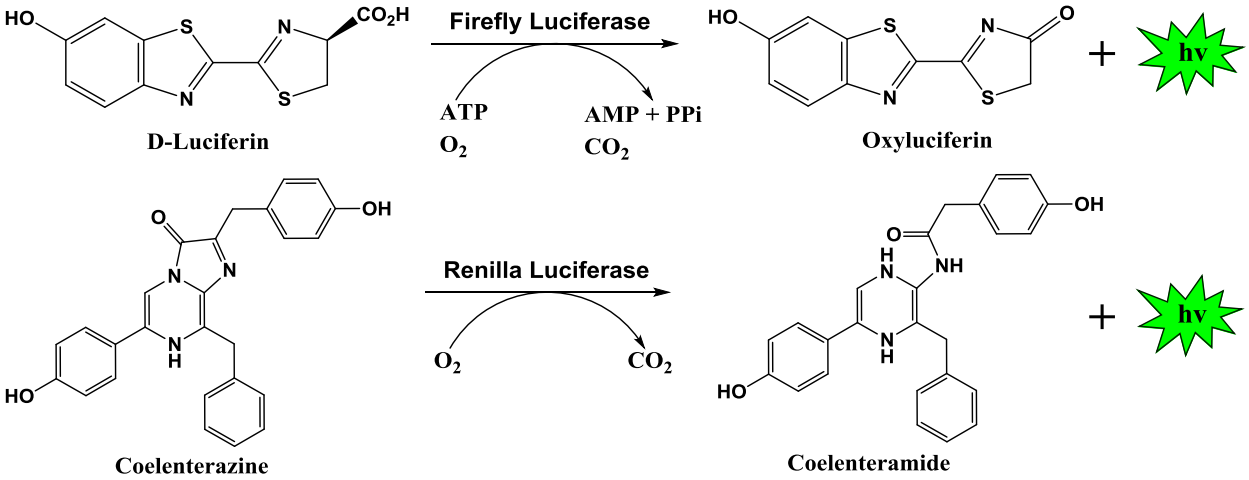

Figure 1. Bioluminescent reactions catalyzed by Firefly and Renilla luciferases.

General Considerations

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.

General Protocol

1. Place the frozen Dual Firefly Luciferase Substrate (Component A), Dual Firefly Luciferase Buffer (Component B), Dual Renilla Luciferase Substrate (Component C) and Dual Renilla Luciferase Buffer (Component D) 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 (Component A) at 1:100 with Dual Firefly Luciferase Buffer (Component B). Mix well by inverting the tube several times.

   For example: If you are running 100 tests, add 100 µL of Dual Firefly Luciferase Substrate (Component A) to 10 mL of Dual Firefly Luciferase Buffer (Component B).
3. Prepare the Dual Renilla Luciferase Assay Reagent by diluting Dual Renilla Luciferase Substrate (Component C) at 1:100 with Dual Renilla Luciferase Buffer (Component D). Mix well by inverting the tube several times.

   For example: If you are running 100 tests, add 100 µL of Dual Renilla Luciferase Substrate (Component C) to 10 mL of Dual Renilla Luciferase Buffer (Component D).
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.