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ABP Firefly Luciferase Assay Kit

Catalog Number: FP304, FP305

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
ABP Firefly Luciferase Assay Kit (Cat. No. FP304)				The product is stable for at least 6 months when stored as directed.
Firefly Luciferase Substrate (Component A)	100 µL	100X	-20 °C Protect from light	
Firefly Luciferase Buffer (Component B)	10 mL	1X		
ABP Firefly Luciferase Assay Kit (Cat. No. FP305)				
Firefly Luciferase Substrate (Component A)	500 µL	100X	-20 °C Protect from light	
Firefly Luciferase Buffer (Component B)	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. Luciferase is a popular choice as a reporter for these applications because functional enzyme is created immediately upon translation and the assay is rapid, reliable and easy to perform. Furthermore, analysis using luciferase as the genetic reporter is well suited to laboratory automation and high-throughput applications.

Firefly luciferase is a cytoplasmic enzyme with a molecular weight of 61 kDa, and catalyzes the mono-oxygenation of luciferin (Figure 1). Luciferin is a relatively stable molecule found only in luminous beetles (including fireflies). The enzyme uses ATP as a co-factor although most of the energy for photon production comes from molecular oxygen. The quantum yield is about 0.9, the highest of any known luminescent reaction. The gene encoding firefly luciferase (*luc*) is a cDNA clone that has been incorporated into a number of reporter vectors.

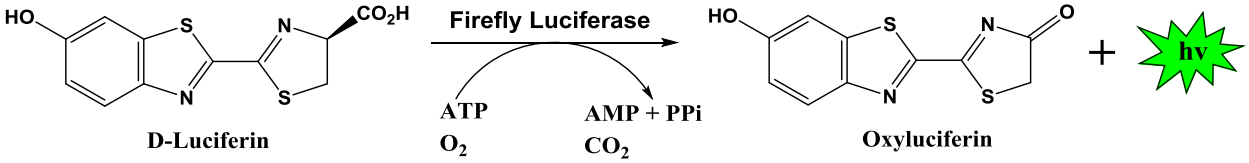

Figure 1. The Firefly luciferase reaction.

ABP Firefly Luciferase Assay Kit provides a simple and robust assay system for measuring Firefly luciferase activities from a single sample. The Firefly Luciferase Substrate and Firefly Luciferase Buffer provided with the kit, are combined to form the Firefly Luciferase Assay Reagent, which is added directly to cells in growth medium without washing or preconditioning. The assay is compatible with general used culture media with signal half-life of ~1 hr. The Firefly Luciferase Assay Reagent is used to lyse cells in culture medium and generate luminescent signal simultaneously 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.

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 Firefly Luciferase Substrate (Component A) and Firefly Luciferase Buffer (Component B) in a water bath at room temperature. Mix well after thawing.
2. Prepare the Firefly Luciferase Assay Reagent by diluting Firefly Luciferase Substrate (Component A) at 1:100 with 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 Firefly Luciferase Substrate (Component A) to 10 mL of Firefly Luciferase Buffer (Component B).
3. 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.
4. Add a volume of reagent equal to that of culture medium in each well. Mix for optimal consistency. For 96-well plates, typically 80 µL of reagent is added to cells grown in 80 µL of medium. For 384-well plates, typically 30 µL of reagent is added to cells grown in 30 µL of medium.
5. Place the culture plates on a rocking platform or orbital shaker with gentle rocking/shaking for ~10 min.
6. Measure the luminescence signal in a luminometer (consult the instrument manual).