Description

The TWO-Step Luciferase (Firefly & Renilla) Assay System is designed for high throughput, rapid quantitation of both Firefly and Renilla luciferases in a single mammalian cell culture sample. It comes with enough reagents for 1000 assays of 100 µl each in a 96-well plate.

This assay system has several relevant features:

- Sensitive – highly sensitive detection of Firefly and Renilla luciferase activity.
- Stable – the luciferase signal output is stable for more than one hour, providing incubation time flexibility.
- High-throughput – its simple homogeneous protocol minimizes handling steps and supports high-throughput screening applications.
- Compatibility – works with a variety of common media containing up to 10% serum and phenol red.

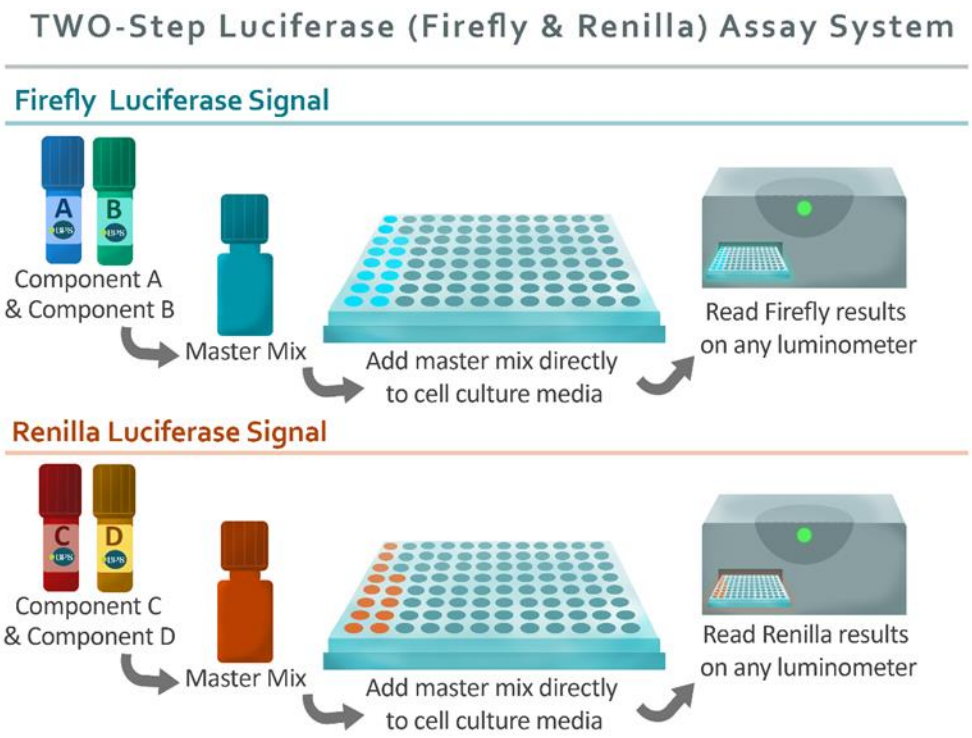

Figure 1: TWO-Step Luciferase (Firefly & Renilla) Assay System protocol overview.

The Firefly Luciferase Reagent is added directly to the cell culture medium. This reagent lyses the cells and contains a substrate that allows Firefly luciferase to generate a luminescence signal. Next, the Renilla Luciferase Reagent is added to the same well. The reagent quenches the Firefly luciferase luminescence and provides the substrate for Renilla luciferase to generate Renilla luciferase luminescence signal. The signal generated by both reactions can be conveniently measured on a luminometer.

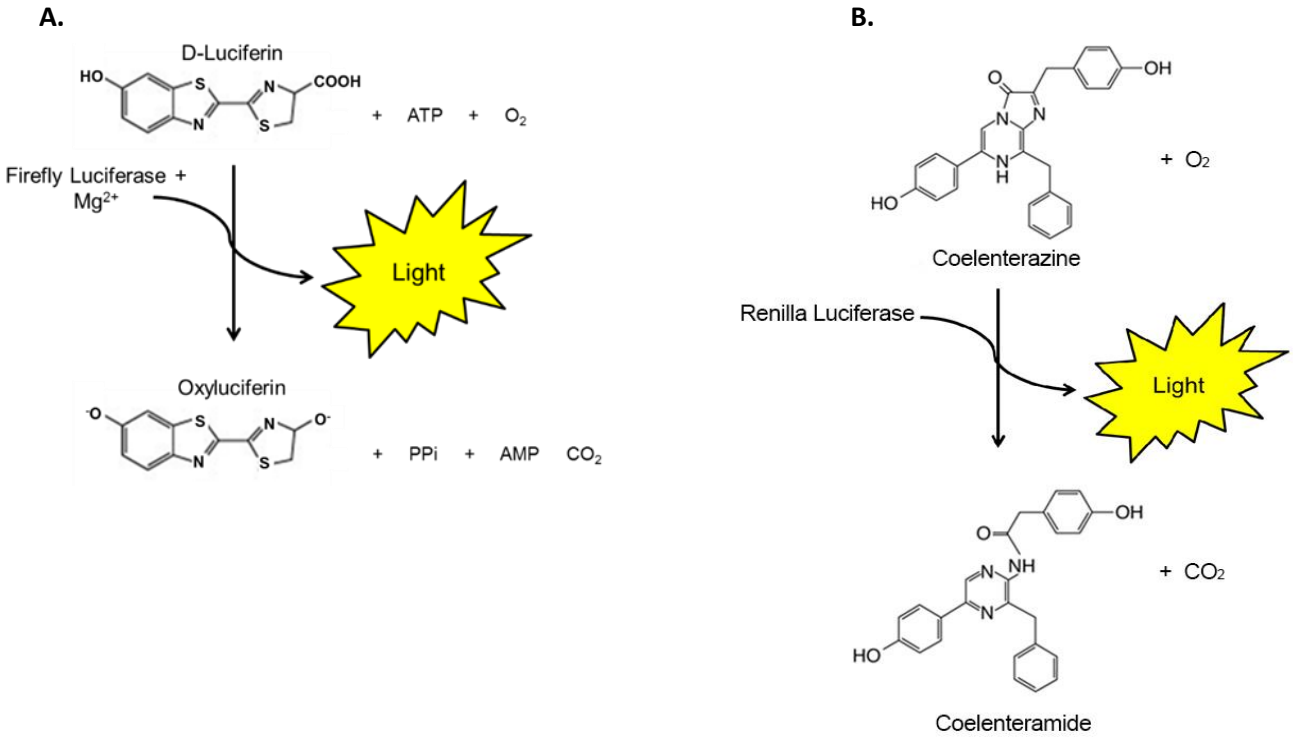

Figure 2: Chemical reactions involved in the TWO-Step Luciferase (Firefly & Renilla) Assay System.
A. Chemical reaction involved in signal generation by Firefly Luciferase Reagent Buffer (Component A) and Firefly Luciferase Reagent Substrate (Component B). **B.** Chemical reaction involved in signal generation by Renilla Luciferase Reagent Buffer (Component C) and Renilla Luciferase Reagent Substrate (Component D).

Background
Firefly luciferase has been used as a sensitive reporter to study a wide range of biological responses. It was first cloned from the North American *Photinus pyralis* and catalyzes the oxidation of D-luciferin, in the presence of ATP and magnesium, emitting yellow light. This reaction has a high quantum yield and both luciferase and luciferin have low toxicity. These characteristics contributed to Firefly luciferase becoming a commonly used tool. Renilla luciferase, on the other hand, is ATP-independent and converts coelenterazine to coelenteramide in the presence of oxygen, emitting blue light. Both luciferases have a relatively low half-life, versus for instance fluorescent proteins, and allow dynamic studies to be performed. The accuracy of Firefly luciferase reporter in response to a certain stimulus can be improved by the normalization to a control reporter, such as Renilla luciferase reporter, in the same sample.

Applications

- Monitor Firefly (*Photinus pyralis*) and Renilla luciferase activity in cultured mammalian cells.
- Luminescence-based high-throughput drug screening.

Supplied Materials

Catalog #	Name	Amount	Storage
	Firefly Luciferase Reagent Buffer (Component A)	100 ml	-20°C
	Firefly Luciferase Reagent Substrate (Component B)	1000 µl	-20°C (Protect from light)
	Renilla Luciferase Reagent Buffer (Component C)	100 ml	Room Temp.
	Renilla Luciferase Reagent Substrate (Component D)	1000 µl	-20°C (Protect from light)

** Renilla Luciferase Reagent Buffer (Component C) should be thawed at 37°C overnight immediately after receipt. It can be stored at Room Temperature indefinitely.*

Materials Required but Not Supplied

- Multiwell tissue culture plates compatible with the reading instrument.
- Mammalian cells express Firefly Luciferase under the experimental promoter and Renilla Luciferase under a control promoter.
- Appropriate cell culture medium.
- Laboratory platform shaker.
- Luminometer.

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

Avoid exposure to excessive heat or light during incubation.

Assay Protocol

- The protocol described is designed for a 96-well format. The use of other formats requires appropriate scaling of volumes and optimization.
- Firefly Luciferase Reagent Buffer (Component A) should be brought to Room Temperature (RT, 20- 25°C) before use.
- Firefly Luciferase Assay Working Solution and Renilla Luciferase Assay Working Solution should be prepared on the day of use.
- When analyzing multiple plates, it is recommended to include a common control sample in each plate and normalize the luminescence of each plate to the control contained in the same plate.
- The assay should include a "Background Control" (cell-free wells) condition since background luminescence is a characteristic of luminometer performance.
- The volume of Firefly Luciferase Assay Working Solution (see preparation mode below) to be used should be the same volume as culture medium present in the cell culture wells.

- The volume of Renilla Luciferase Assay Working Solution (see preparation mode below) to be used should be the same volume as culture medium present in the cell culture wells.

1. Thaw **Firefly Luciferase Reagent Buffer (Component A)** by placing the reagent in a water bath at RT.

2. Equilibrate the buffer to RT and mix well before use.

3. Immediately prior to performing the experiment, prepare adequate volume of **Firefly Luciferase Assay Working Solution** (equal volume/well as cell culture medium volume present in the well) by diluting **Firefly Luciferase Reagent Substrate (Component B)** 100-fold with **Firefly Luciferase Reagent Buffer** (**Component A)** and mix well.

Note: Avoid exposure to excessive light.

Note: Store remaining Firefly Luciferase Reagent Buffer (Component A) and Firefly Luciferase Reagent Substrate (Component B) separately at -20°C.

4. Add equal volume/well of Firefly Luciferase Assay Working Solution as the cell culture medium volume present in the well, including to the "Background Control" wells (cell-free wells).

5. Gently rock the plates for ≥15 minutes at RT.

6. Measure the luminescence generated by Firefly luciferase using a luminometer.

Note: The signal under these conditions will be stable for more than 2 hours at RT. For maximal light intensity, measure samples within 1 hour of reagent addition.

7. Prepare adequate volume of **Renilla Luciferase Assay Working Solution** (equal volume/well as cell culture medium volume present in the well) by diluting **Renilla Luciferase Reagent Substrate (Component D)** 100-fold with **Renilla Luciferase Reagent Buffer (Component C)** and mix well.

Note: Avoid exposure to excessive light.

Note: Store remaining Renilla Luciferase Reagent Buffer (Component C) at RT and Renilla Luciferase Reagent Substrate (Component D) -20°C.

8. Add equal volume/well of Renilla Luciferase Assay Working Solution as the cell culture medium volume present in the well, including to the "Background Control" wells (cell-free wells).
Example: A 96-well plate with 100 µl of culture medium per well will take 100 µl of Firefly Luciferase Assay Working Solution/well and 100 µl of Renilla Luciferase Assay Working Solution/well.

9. Agitate the plates very gently, to avoid liquid spill, for ~1 minute a RT.

10. Measure the Renilla luminescence signal using a luminometer.

11. The "Background Control" luminescence should be subtracted from all other readings.

Example Results

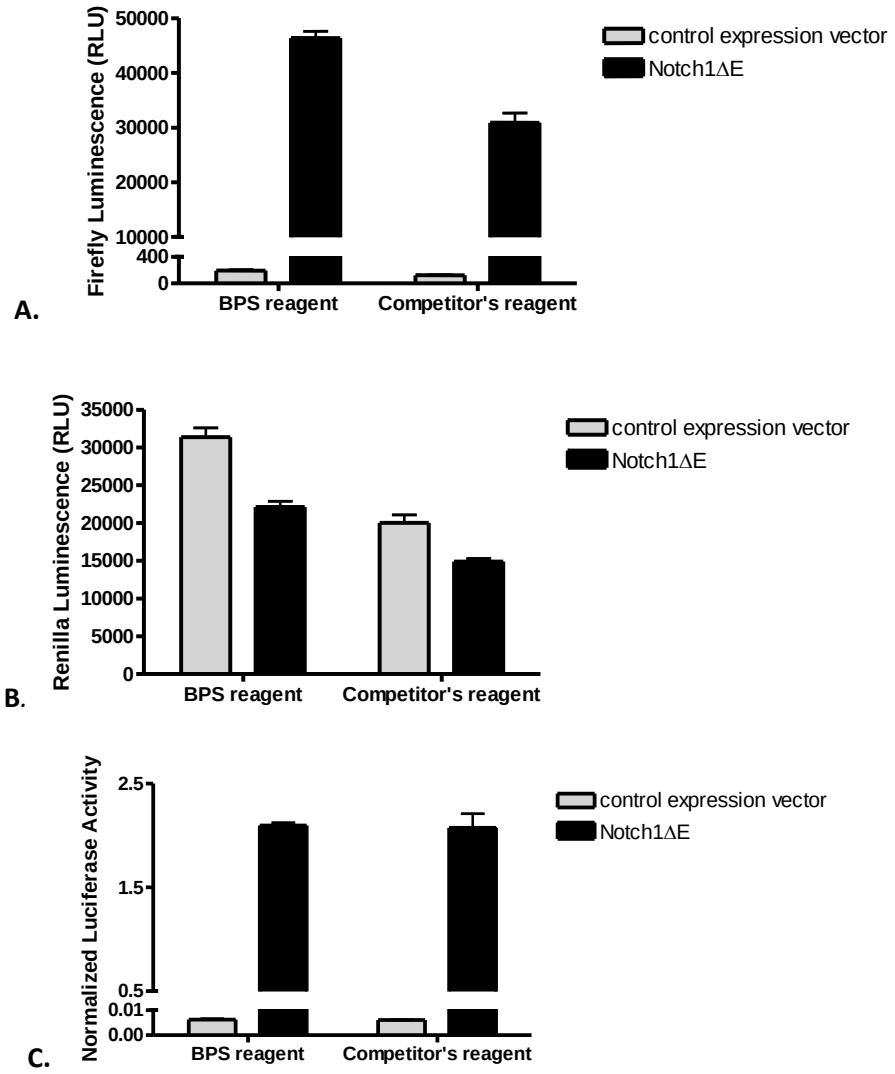

Figure 1: Luciferase activity measured with TWO-Step Luciferase (Firefly & Renilla) Assay System and another commercially available luciferase reagent.
HEK293 cells were seeded in a 96-well plate and transfected with a Notch Firefly luciferase reporter, a constitutively expressing Renilla luciferase vector, and a Notch1 expression vector or control expression vector (Mouse Notch1 Pathway Reporter Kit BPS Bioscience #60509). 48 hours post-transfection, luminescence was measured with TWO-Step Luciferase (Firefly & Renilla) Assay System or another commercially available option. Luciferase reagents were added to the cells and luminescence was measured 15 minutes after reagent addition. Data shown represents background-subtracted luminescence. **A.** Analysis of Firefly-derived luminescence signals generated by Notch Firefly luciferase measured with BPS Bioscience or a competitor reagent. **B.** Analysis of Renilla-derived luminescence signal measured with BPS Bioscience or a competitor reagent. **C.** Normalized luciferase activity, corresponding to the ratio of Firefly-derived luminescence from Notch Firefly luciferase to the Renilla-derived luminescence generated by control Renilla luciferase.

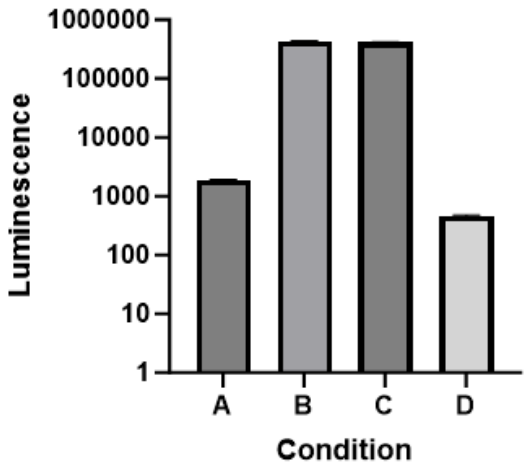

Figure 2: The TWO-Step Luciferase (Firefly & Renilla) Assay System component Renilla Luciferase Assay Working Solution can completely quench Firefly-derived luminescence.
Firefly Luciferase HL-60 cells (no Renilla luciferase is expressed in this cell line) were seeded at 60k cells per well in 100 µl of cell culture medium. Wells containing only medium were used as control (A). Firefly luciferase enzyme activity was measured by adding Firefly Luciferase Assay Working Solution (B). To determine the quenching ability of the buffer system, present in Renilla Luciferase Assay Working Solution, 100 µl of cell culture medium (C) or Renilla Luciferase Assay Working Solution (D) were added to each well 5 minutes after the first reading. The quenching percentage of Renilla Luciferase Assay Working Solution was 99.87% as calculated by the formula: 1 – [(Average luminescence value after quenching)/ (Average luminescence before quenching)] *100.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
ONE-Step™ Luciferase Assay System	60690	10 ml/100 ml/500 ml/1 L
Firefly Luciferase Lentivirus (G418, Hygromycin and Puromycin)	79692	500 µl x 2
Renilla Luciferase Lentivirus (G418 and Puromycin)	79565	500 µl x 2
Firefly Luciferase Lentivirus (EF1A Promoter/ Geneticin, Hygromycin and Puromycin)	78740	500 µl x 2
Firefly Luciferase-eGFP Lentivirus (EF1A Promoter/ Geneticin, Hygromycin and Puromycin)	78741	500 µl x 2
Luciferase, His-Tag (Firefly) Recombinant	100576	100 µg/500 µg/1 mg

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