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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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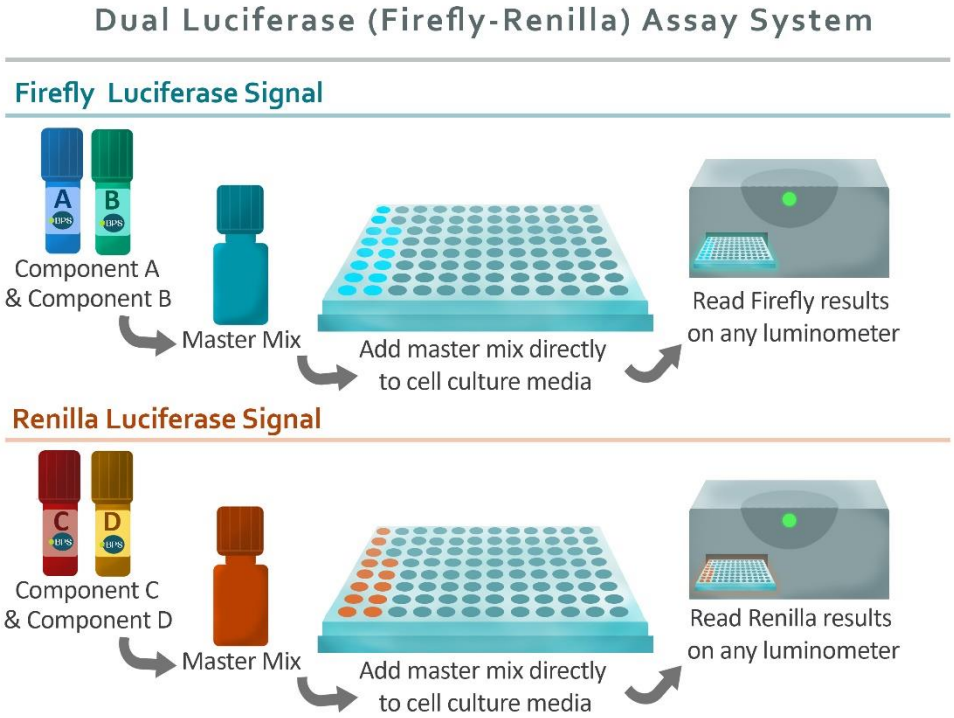
Data Sheet

Dual Luciferase (Firefly-Renilla) Assay System
Catalog #: 60683-3
Size: 1 L

Description

Firefly luciferase has been used as a sensitive reporter to study a wide range of biological responses. However the change of the expression of Firefly luciferase reporter can be due to a global effect instead of a specific effect. The accuracy of Firefly luciferase reporter can be improved by normalizing to a control reporter, such as Renilla luciferase reporter, in the same sample.

The ***Dual Luciferase (Firefly-Renilla) Assay System*** is designed to be used for high-throughput, rapid quantitation of both Firefly and Renilla luciferases from a single sample in mammalian cell culture. The Firefly Luciferase Reagent is first added to the cells in medium directly. This reagent lyses the cells and contains a substrate for firefly luciferase to produce firefly luciferase luminescence. Next, the Renilla Luciferase Reagent is added to the same well. It quenches the firefly luciferase luminescence and provides the substrate for renilla luciferase to produce renilla luciferase luminescence. The light production of both reactions can be conveniently measured on a luminometer.



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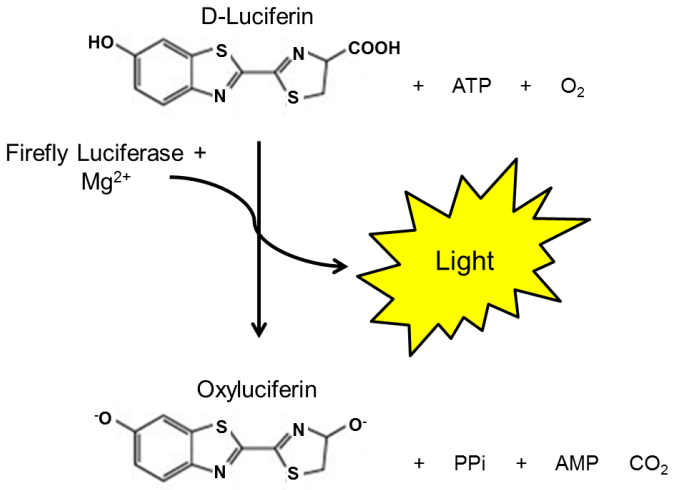
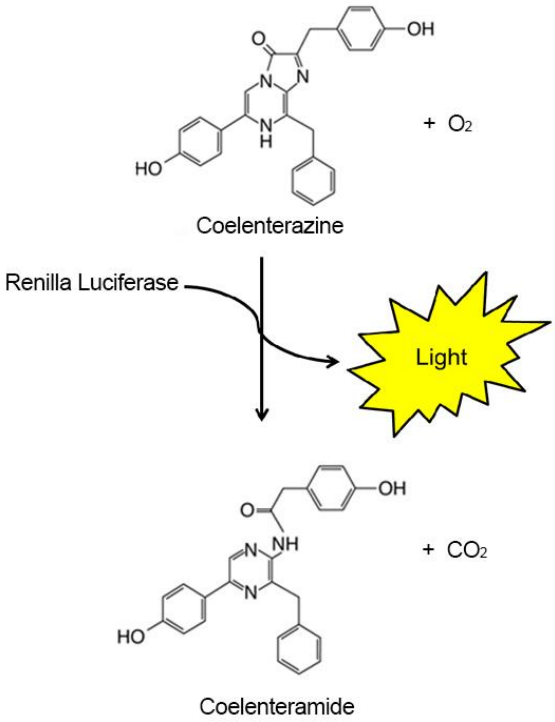
Reaction Schemes:

Component A & Component B



Component C & Component D



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This assay system has several features:

- Sensitive – highly sensitive detection of firefly luciferase activity and Renilla luciferase activity.
- Stable – the luciferase signal output is stable for more than one hour, providing flexibility with regard to incubation time
- High-throughput – simple homogeneous protocol minimizes handling steps to support high-throughput screening applications
- Compatibility – works well with a variety of common media containing 0-10% serum and phenol red.

Application

- Monitor firefly (*Photinus pyralis*) and Renilla luciferase activity in cultured mammalian cells.
- High-throughput drug screening using Firefly-Renilla dual luciferase reporter.

Components

Component	Amount	Storage
Firefly Luciferase Reagent Buffer (**Component A**)	10 x 100 ml	-20°C
Firefly Luciferase Reagent Substrate 100x (**Component B**)	10 x 1000 µl	-20°C *Protect from light*
Renilla Luciferase Reagent Buffer (**Component C**)	10 x 100 ml	Room Temp.
Renilla Luciferase Reagent substrate 100x **(Component D**)	10 x 1000 µl	-20°C *Protect from light*

Each system contains sufficient reagents to perform 1000 assays of 100 µl each in 96-well plate.

Stability

At least 6 months when stored as directed. Upon first thaw, store **Components A, B, and D** at -20°C. Store the **Component C** at room temperature. The reagent may be subjected to several freeze/thaw cycles with no effect on functionality, but it is recommended that freeze/thaw cycles be avoided whenever possible.

Important Product Information

- The reagent has been validated in a 96-well format. Other formats will require scaling and optimization by the end-user.

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- Firefly Luciferase Reagent Buffer (Component A) must be at ~ room temperature (20-25°C) before use.
- Prepare Firefly Luciferase Assay Working Solution and Renilla Luciferase Assay Working Solution on the day it is to be used.
- Firefly Luciferase Assay Working Solution should be added to cells for at least 5 minutes before measuring luminescence to allow complete cell lysis.
- For maximal light intensity, measure samples within 1 hour of reagent addition.
- Avoid exposing to excessive heat or light during incubation.
- Different cell lines may exhibit variation in lysis ability and/or luminescence signal and may require slight optimization by the end-user.
- Luminescence signal is affected by assay conditions. Results should be compared between samples measured using the same cell type and media/serum combinations.
- To analyze multiple plates, include a common control sample in each plate and normalize the luminescence of each plate to the control contained in the same plate.
- Background luminescence is a characteristic of luminometer performance, therefore, background luminescence must be subtracted from all readings for accuracy.

Materials Required but Not Supplied

- Multiwell tissue culture plates that are compatible with luminometer being used
- Mammalian cells that express Firefly Luciferase and Renilla Luciferase
- Appropriate cell culture medium
- Laboratory platform shaker
- Luminometer

General Assay Procedure

1. Thaw Firefly Luciferase Reagent Buffer (**Component A**) by placing the reagent in a room temperature water bath. Equilibrate the buffer to room temperature and mix well before use.
2. Calculate the amount of Firefly Luciferase Assay Working solution needed for the experiment (**Component A** + **Component B**). Immediately prior to performing the experiment, prepare the Firefly Luciferase Assay Working Solution by diluting Firefly Luciferase Reagent Substrate (**Component B)** into Firefly Luciferase Reagent Buffer (**Component A)** at a 1:100 ratio and mix well. Avoid exposing to excessive light*. Only use enough of each component for the experiment, remaining **Component A** and **Component B** should be stored separately at* -20°C*.*
3. Remove multi-well plate containing mammalian cells from incubator. *Note: plates must be compatible with luminescence measurement with luminometer being used.*
4. Add equal volume of Firefly Luciferase Assay Working Solution (**Component A** + **Component B**) to the culture medium in each well. Example: 96-well plate with 100 µl of culture medium requires 100 µl of Firefly Luciferase Assay Working Solution per well.

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5. Gently rock the plates for ≥15 minutes at room temperature. Measure firefly luminescence using a luminometer.

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

6. Calculate the amount of Renilla Luciferase Assay Working Solution needed for the experiment (**Component C** + **Component D**). Prepare the Renilla Luciferase Assay Working Solution by diluting Renilla Luciferase Reagent Substrate (**Component D)** into Renilla Luciferase Reagent Buffer (**Component C)** at a 1:100 ratio and mix well. Avoid exposing to excessive light*. Only use enough of each component for the experiment,*

7. Add equal volume of Renilla Luciferase Assay Working Solution (**Component C** + **Component D**) to each well. Example: 96-well plate with 100 µl of culture medium + 100 µl Firefly Luciferase Reagent requires 100 µl of Renilla Luciferase Assay Working Solution per well.

8. Gently rock the plates for ~1 minute at room temperature. Measure renilla luminescence using a luminometer.

9. Data analysis: subtract background (wells with medium and luciferase reagent only) from all the readings.

Figure 1 Comparison of Dual Luciferase (Firefly-Renilla) Assay System to another commercially available luciferase reagent.

HEK293 cells were seeded in a 96-well plate and transfected with Notch firefly luciferase reporter, constitutively expressing Renilla luciferase vector, and Notch1 expression vector or control expression vector (BPS cat # 60509). After ~48 hours of transfection, perform dual luciferase assay. Luciferase reagents were added to the cells and luminescence was measured 15 minutes after reagent addition. Data are shown as background-subtracted luminescence.

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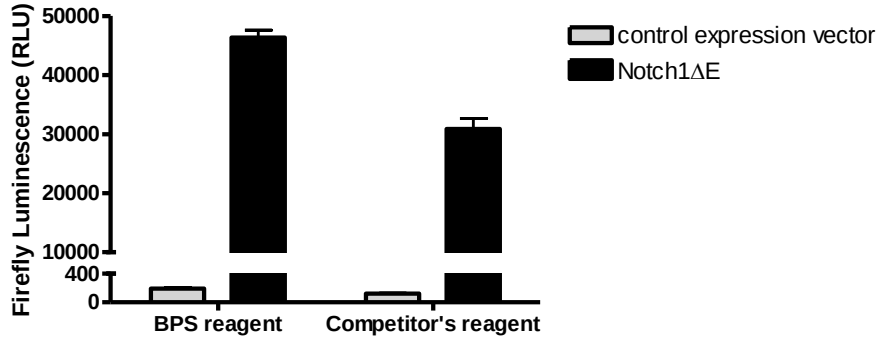
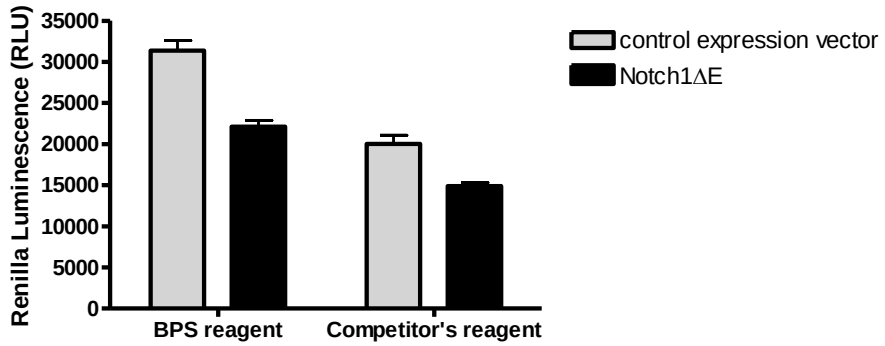
1A. Firefly luminescence from Notch firefly luciferase



1B. Renilla luminescence from control Renilla luciferase



1C. Normalized luciferase activity: ratio of Firefly luminescence from Notch firefly luciferase to Renilla luminescence from control Renilla luciferase.

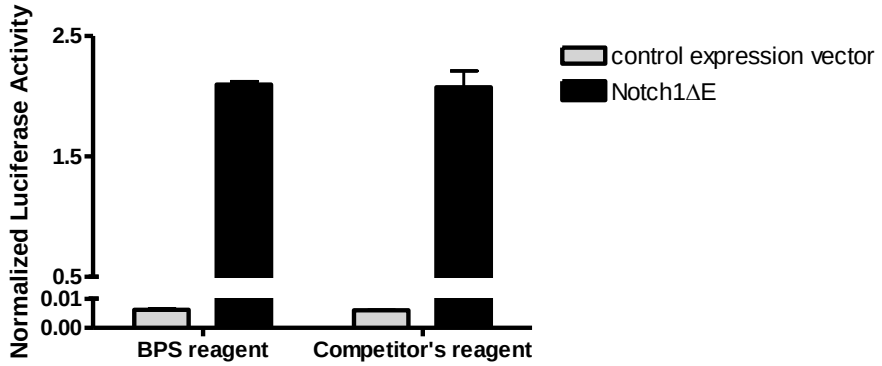


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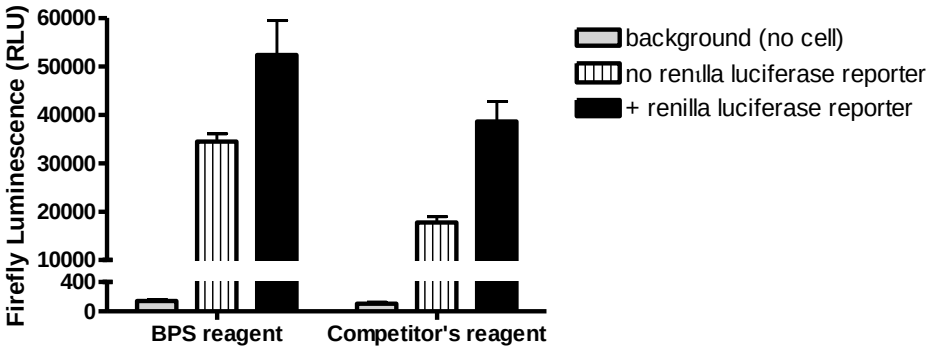
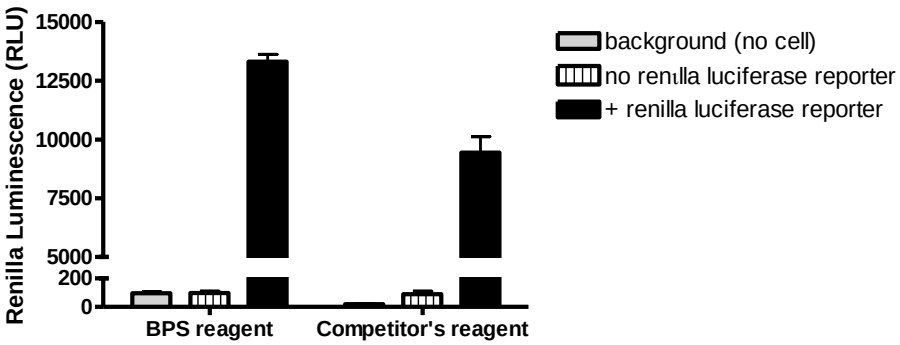
Figure 2 Renilla luciferase reagent completely quench the firefly luciferase luminescence

HEK293 cells were seeded in a 96-well plate and transfected with Notch firefly luciferase reporter and Notch1 expression vector with or without constitutively expressing Renilla luciferase vector (BPS cat # 60509). After ~48 hours of transfection, perform dual luciferase assay.

2A. Firefly luminescence from Notch firefly luciferase



2B. Renilla luminescence from control Renilla luciferase



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Related Products:

Product	Cat. #	Size
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100ml
ONE-Step™ Luciferase Assay System	60690-3	1 L
SBE Reporter Kit	60654	500 rxns.
Myc Reporter Kit	60519	500 rxns.
ARE Reporter Kit	60514	500 rxns.
SBE Reporter Kit	60654	500 rxns.
SRE Reporter Kit	60511	500 rxns.
NF-κB Reporter Kit	60614	500 rxns.
ISRE Reporter Kit	60613	500 rxns.
CRE/CREB Reporter Kit	60611	500 rxns.
FOXO Reporter Kit	60643	500 rxns.
AP1 Reporter Kit	60612	500 rxns.
Notch1/CSL Reporter Kit	60509	500 rxns.
GAL4 Reporter Kit	60522	500 rxns.
TCF/LEF Reporter Kit	60500	500 rxns