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Data Sheet

One-Step Luciferase Assay System
Catalog #: w70701-1
Size: 10 ml

Description

The One-Step Luciferase Assay System is designed to be used for high-throughput, sensitive quantitation of firefly luciferase activity in mammalian cell culture. The reagent consists of two components, a Luciferase Reagent Buffer (**Component A**) and Luciferase Reagent Substrate (**Component B**). **Component A** and **Component B** are combined to form a working solution that contains all the necessary components for cell lysis and luciferase quantitation. This assay system has several features:

- Sensitive – highly sensitive detection of firefly luciferase activity.
- Stable – the signal output is stable for more than two hours, providing flexibility with regard to incubation time
- Convenient – simple one-step, homogenous protocol.
- High-throughput – one-step homogenous 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.
- Instrumentation – does not require a luminometer with injectors.

Application

- Monitor firefly (*Photinus pyralis*) luciferase activity in cultured mammalian cells.
- High-throughput screening using luciferase reporter cell lines

Components

Component	Amount	Storage
Luciferase Reagent Buffer (**Component A**)	2 x 5 ml	-20℃
Luciferase Reagent Substrate, 100x (**Component B**)	100 µl	-20℃ *Protect from light*

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

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Stability

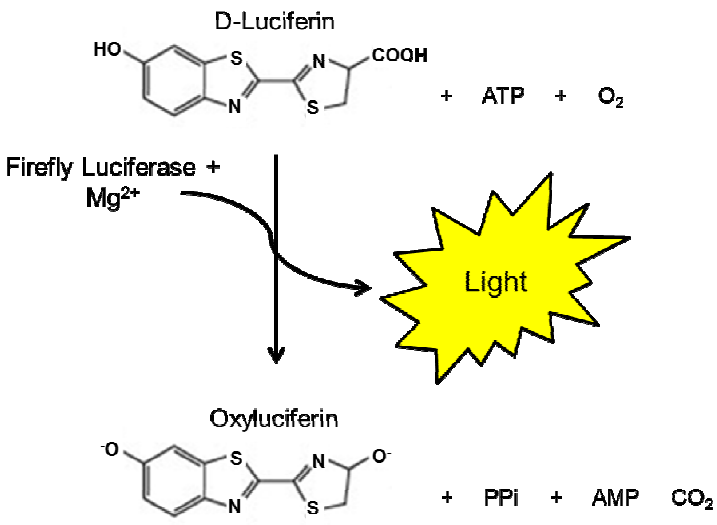
At least 6 months when stored as directed. Upon first thaw, store in aliquots at -20°C. The reagent may be subjected to several freeze/thaw cycles with no effect on functionality, but it is recommended that freeze/thaw cycles be avoided whenever possible.

Background

Luciferase is the general term given to a class of oxidative enzymes that catalyze reactions that give off light, a process known as bioluminescence (Fig. 1). In biology, researchers can take advantage of this reaction and use it as a readout for various biological processes. This has perhaps been exploited most in luciferase reporter cell lines where a promoter region from a gene of interest is placed immediately upstream of the coding sequence for luciferase. In this system, transcriptional activation of the gene of interest leads to a level of luciferase expression that is proportional to the level of gene activation.

The level of activation is then assessed by lysing the cells and adding the luciferase substrate, D-luciferin. Using the One-Step Luciferase Assay System from West Bioscience, this is accomplished in a single step as the lysis buffer has been formulated with D-luciferin. After a brief incubation period, bioluminescence is read on a luminometer.

Figure 1. Bioluminescent reaction catalyzed by luciferase. In the luciferase reaction, the cells are lysed with luciferase substrate containing D-luciferin. Firefly luciferase derived from reporter cells, using ATP and Mg^{2+} as a co-substrate, derived from catalyzes the conversion of D-luciferin to oxyluciferin in a reaction that gives off light. The amount of light given off is proportional to the amount of luciferase present in the reaction and thus, correlates with gene activation. Luminescence is read on a luminometer.



Important Product Information

- The reagent has been validated in a 96-well format. Other formats will require scaling and optimization by the end-user.
- Luciferase Reagent Buffer must be at ~ room temperature (20- 25°C) before use.

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- Luciferase Assay Reagent 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
- Appropriate cell culture medium
- Laboratory platform shaker
- Luminometer

General Assay Procedure

1. Thaw 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 Luciferase Reagent needed for the experiment (**Component A** + **Component B**). Immediately prior to performing the experiment, prepare the luciferase assay working solution by diluting Luciferase Reagent Substrate (**Component B)** into 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℃*.*
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 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 luciferase assay working solution per well.
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.

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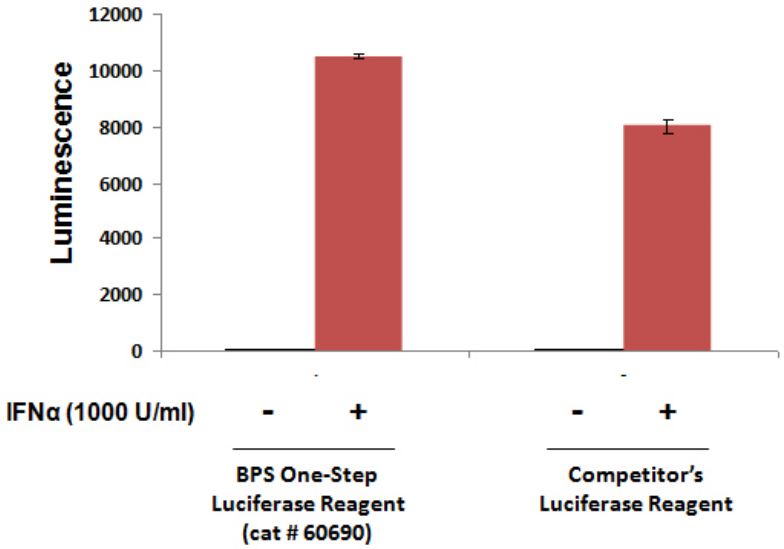
Figure 2. Comparison of One-Step Luciferase Assay System to another commercially available luciferase reagent. JAK/STAT pathway ISRE Reporter – HEK293 Cells (WestBio Cat. # w70521) were seeded in a 96-well plate and treated with or without IFNα to activate the JAK/STAT pathway. The next day, luciferase reagents were added to the cells and luminescence was measured 15 minutes after reagent addition. Data are shown as background-subtracted luminescence.



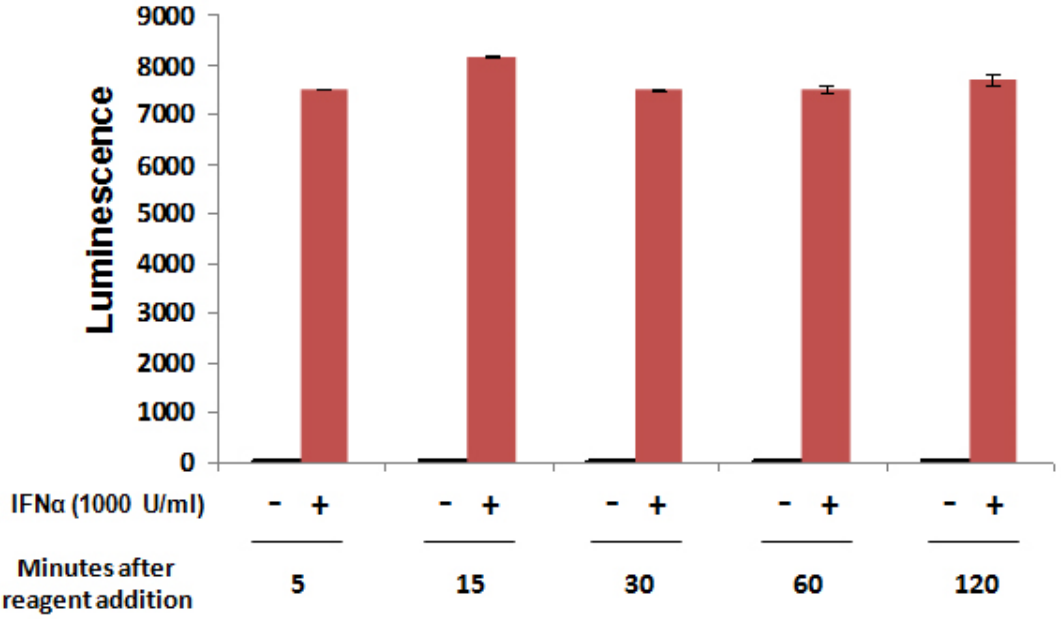


Figure 3. One-Step Luciferase Assay System generates bright luminescence that is stable for hours in luciferase-reporter cells. JAK/STAT pathway ISRE Reporter – HEK293 Cells(WestBio Cat. #w70521) were seeded ina 96-well plate and treated with or without IFNα.The next day, luciferase reagents were added to the cells and luminescence was measured from 5 minutes to 2 hours after reagent addition. Data are shown as background-subtracted luminescence.

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