Description

The One-Step™ Luciferase Assay System is designed 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 reagents for cell lysis and luciferase quantitation.

- Sensitive: highly sensitive detection of firefly luciferase activity.
- Stable: the signal is stable for more than two hours, providing flexibility regarding incubation time.
- Convenient: simple one-step, homogeneous protocol.
- High-throughput: the one-step 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.

Background

Luciferase is the general term given to a class of oxidative enzymes which catalyze reactions that give off light, a process known as bioluminescence (Figure 1)..

Enzymatic activity is directly proportional to the expression of luciferase in the cells and can be quantified upon addition of the appropriate substrate.

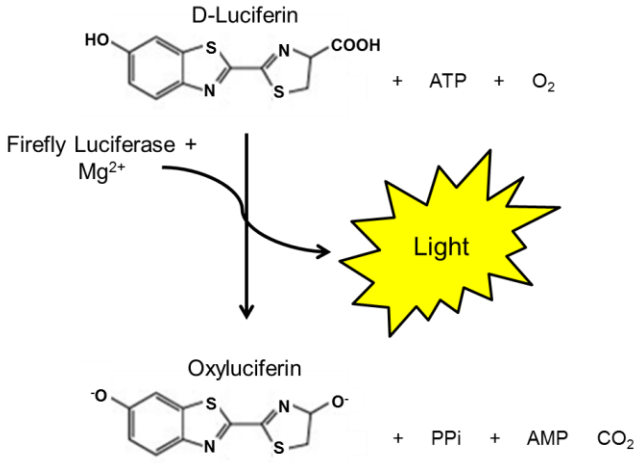


Figure 1. Bioluminescent reaction catalyzed by luciferase.
In the luciferase reaction, the cells are lysed with luciferase substrate containing D-luciferin. Firefly luciferase expressed in reporter cells catalyzes the conversion of D-luciferin to oxyluciferin using ATP as a co-substrate, and in the presence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

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
78262-1	Luciferase Reagent Buffer (**Component A**)	5 x 100 ml	-20°C
78263-1	Luciferase Reagent Substrate, 100x (**Component B**)	5 x 1 ml	-20°C *Protect from light*

Each kit contains sufficient reagents to perform 5000 assays of 100 µl each in 96-well plates.

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.
- 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.
- 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

- Clear-bottom, white multiwell tissue culture plates that are compatible with the luminometer being used
- Mammalian cells that express firefly luciferase
- Appropriate cell culture medium
- Laboratory platform shaker
- Luminometer

Storage Conditions

Reagents will perform optimally for up to 6 months from date of receipt when the materials are 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.

Safety

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

General Assay Protocol

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 before 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.

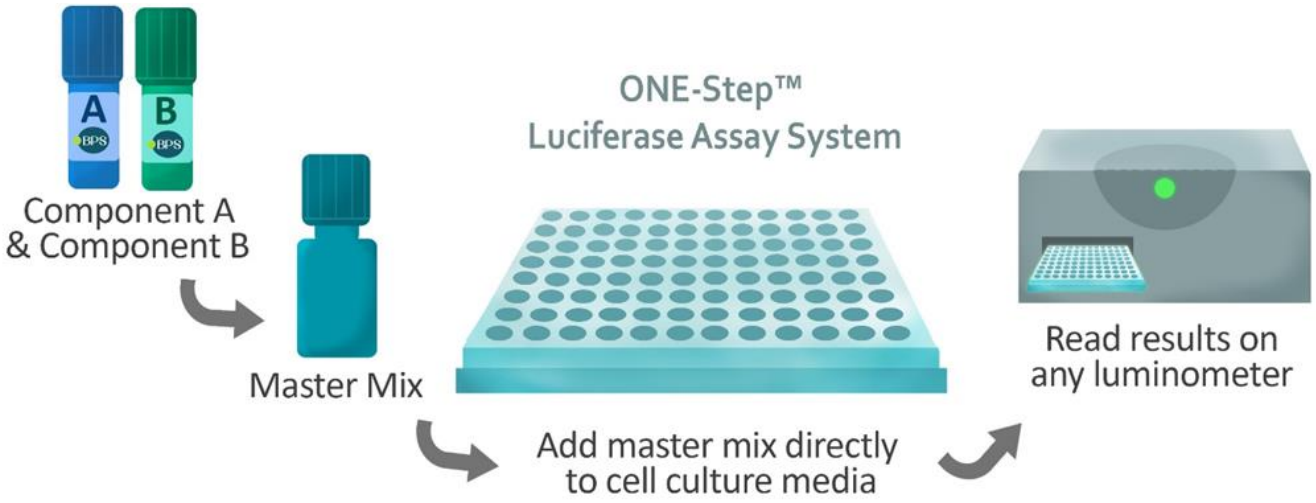
   Avoid exposing to excessive light. Only use enough of each component for the experiment, and store the remaining Component A and Component B separately at -20°C.

3. Remove the plate containing the luciferase-expressing cells from the incubator. Note: the plates must be compatible with the luminometer being used.

4. Add the luciferase assay working solution (**Component A + Component B**) directly to the culture medium using an equal volume as the volume of the culture medium. For example: 96-well plate containing 100 µl of culture medium/well requires 100 µl/well of luciferase assay working solution.

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

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



Example of Assay Results

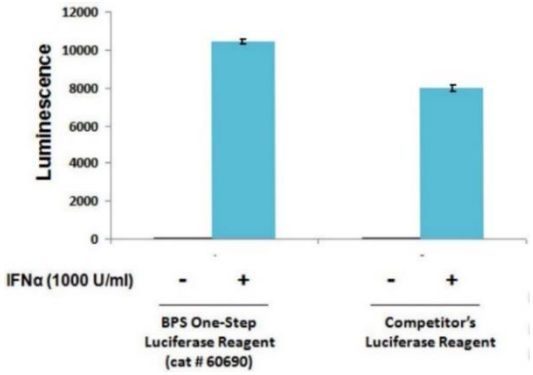


Figure 2: Comparison of ONE-Step™ Luciferase Assay System to another commercially available luciferase reagent. JAK/STAT pathway ISRE Reporter HEK293 Cells (BPS Bioscience #60510) were seeded in a 96-well plate and treated with or without IFNα to activate the JAK/STAT pathway. The next day, luciferase reagents were added to the cells and luminescence was measured 15 minutes after reagent addition. Data are shown as background-subtracted luminescence.

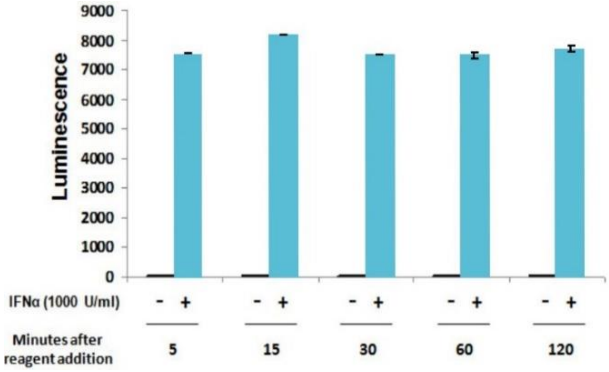


Figure 3: ONE-Step™ Luciferase Assay System generates bright luminescence that is stable for hours in luciferase-reporter cells.
JAK/STAT pathway ISRE Reporter HEK293 Cells (BPS Bioscience #60510) were seeded in a 96-well plate and treated with or without IFNα. The next day, luciferase reagents were added to the cells and luminescence was measured from 5 minutes to 2 hours after reagent addition. Data are shown as background-subtracted luminescence.

Related Products

Products	*Catalog #*	*Size*
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683-1	10 ml
NF-κB reporter (Luc) - HEK293 Recombinant Cell line	60650	2 vials
PD-1/NFAT Reporter Jurkat Recombinant Cell Line	60535	2 vials
Firefly Luciferase THP-1 Cell Line	78409	2 vials
Firefly Luciferase NALM6 Cell Line	78494	2 vials
Firefly Luciferase Raji Cell Line	78622	2 vials