Amplite™ Luciferase Reporter Gene Assay Kit
Bright Glow

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 12518 (1 plate); 12519 (10 plates); 12520 (100 plates)	Keep in freezer Avoid exposure to light	Luminescence microplate readers

Introduction

Common reporter genes include β-galactosidase, β -glucuronidase and luciferase. The advantages of a luciferase assay include: high sensitivity, absence of luciferase activity inside most of the cell types, wide dynamic range, speed and low cost. The most versatile and common reporter gene is the luciferase of the North American firefly photinus pyralis. The protein requires no posttranslational modification for enzyme activity. It is not even toxic in high concentration (*in vivo*) and can be used in pro- and eukaryotic cells. The firefly luciferase catalyzes the bioluminescent oxidation of luciferin in the presence of ATP, magnesium and oxygen. This Amplite™ Luciferase Reporter Gene Assay Kit uses a proprietary luminogenic formulation to quantify luciferase activity in live cells and cell extracts. Our formulation generates a luminescent product that gives strong luminescence upon interaction with luciferase. The kit provides all the essential components with our optimized "mix and read" assay protocol that is compatible with HTS liquid handling instruments. It has high sensitivity, and can be used for the assays that require low detection limit.

The kit provides a fast, simple, and homogeneous bioluminescence assay for studying gene regulation and function. This assay is based on firefly luciferase, a monomeric 61 kD enzyme that catalyses a two-step oxidation of luciferin, which yields light at 560 nm. The first step involves the activation of the protein by ATP to produce a reactive mixed anhydride intermediate. In the second step, the active intermediate reacts with oxygen to create a transient dioxetane, which quickly breaks down to the oxidized products, oxyluciferin and carbon dioxide, along with a burst of light. The firefly luciferase is a very sensitive genetic reporter due to the lack of any endogenous activity in mammalian cells. This reaction is very efficient and the quantum yield is the highest of any characterized bioluminescent reaction. The assay can be performed in a convenient 96-well and 384-well microtiter-plate format. The "glow-type" signal with a half-life of two to four hours provides a consistent signal across large batches of plates. The assay is compatible with the use of standard cell growth media.

$$\text{Luciferin} + \text{ATP} + O_2 \xrightarrow{Mg^{2+}} \text{Oxyluciferin} + \text{AMP} + \text{Pyrophosphate} + CO_2 + \text{light}\ (\sim 560\ \text{nm})$$

Kit Key Features

Sensitive:	Detect as low as 0.1 pg luciferase/well.
Continuous:	Stable luminescence, suitable for manual or automated operations without a mixing or separation step.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	**Amount**		
	Cat. # 12518 (1 plate)	**Cat. # 12519** (10 plates)	**Cat. # 12520** (100 plates)
Component A: Luciferase Sensor (Light-sensitive)	1 bottle	1 bottle	1 bottle
Component B: Assay Buffer	1 bottle (10 mL)	1 bottle (100 mL)	1 bottle (1000 mL)

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Assay Protocol for One 96-well Plate

Brief Summary

Prepare cells (samples) with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Add equal volume of luciferase assay solution → Incubate at room temperature for 10-20 minutes → Monitor luminescence intensity

1. Prepare cells (or samples):

1.1 For adherent cells: Plate cells overnight in growth medium at 1,000 -10,000 cells/90 µL/well (96-well plate) or 250-2,000 cells/20 µL/well (384-well plate).

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 2,000-20,000 cells/90 µL/well for a 96-well poly-D lysine plate or 500-5,000 cells/20 µL/well for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiment.
Note 1: Each cell line should be evaluated on an individual basis to determine the optimal cell density. Cells may be seeded the day before or on the day of the experiment depending upon the cell type and/or the effect of the test compounds.
Note 2: For all luminescent experiments, it is recommended to use white plates to get the best results.

2. Prepare luciferase assay solution:

2.1 Thaw all the kit components to room temperature before use.

2.2 For Cat. # 12518, transfer the whole content of Reaction Buffer (Component B) into the bottle of Luciferase Sensor (Component A), and mix well.

For Cat. # 12519, add 10 mL of Reaction Buffer (Component B) and for Cat. # 12520, add 100 mL of Reaction Buffer (Component B) into the bottle of Luciferase Sensor (Component A), and mix well. Transfer the resulted solution back to the bottle of Reaction Buffer (Component B). Multiple washes are necessary to completely transfer the contents.
Note: The reconstituted luciferase assay solution is not stable. Aliquot and store the unused reconstituted luciferase assay solution at -80 ^{o}C. Avoid freeze/thaw cycles.

3. Run luciferase assay:

3.1 Treat cells (or samples) with test compounds by adding 10 µL of 10X test compounds (96-well plate) or 5 µL of 5X test compounds (384-well plate) in desired compound buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.

3.2 Incubate the cell plate in a 5% CO_2 incubator at 37 ^{o}C for desired period of time, typically 4 hours to overnight.

3.3 Add 100 µL (96-well plate) or 25 µL (384-well plate) per well of luciferase assay solution (from Step 2.2) and incubate the plate at room temperature for 10-20 minutes. Keep from light.

3.4 Monitor luminescence intensity with a luminometer.

4. Establish standard luciferase calibration curve:

Luciferase standard curve should be generated together with the above assay if the absolute amount of luciferase in samples needs to be calculated.

4.1 Make a series dilutions of luciferase in PBS buffer with 0.1% BSA by including a sample without luciferase (as a control) for measuring background luminescence.
Note: Typically luciferase concentrations from 1 pg/mL to 1 ng/mL are appropriate.

4.2 Add the same amount of the diluted luciferase solution into an empty plate (100 µL for a 96-well plate, 25 µL for a 384-well plate).

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4.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of luciferase assay solution (from Step 2.2).

4.4 Incubate the reaction mixture at room temperature for 10-20 minutes, kept from light.

4.5 Record the luminescence intensity with a standard luminometer.

4.6 Generate the luciferase standard curve.

Data Analysis

The luminescence in blank wells with the growth medium is used as a control, and is subtracted from the values for the cell (or sample) wells. The background luminescence of the blank wells may vary depending on the sources of the growth media or the microtiter plates. A luciferase titration curve is shown in Figures 1.

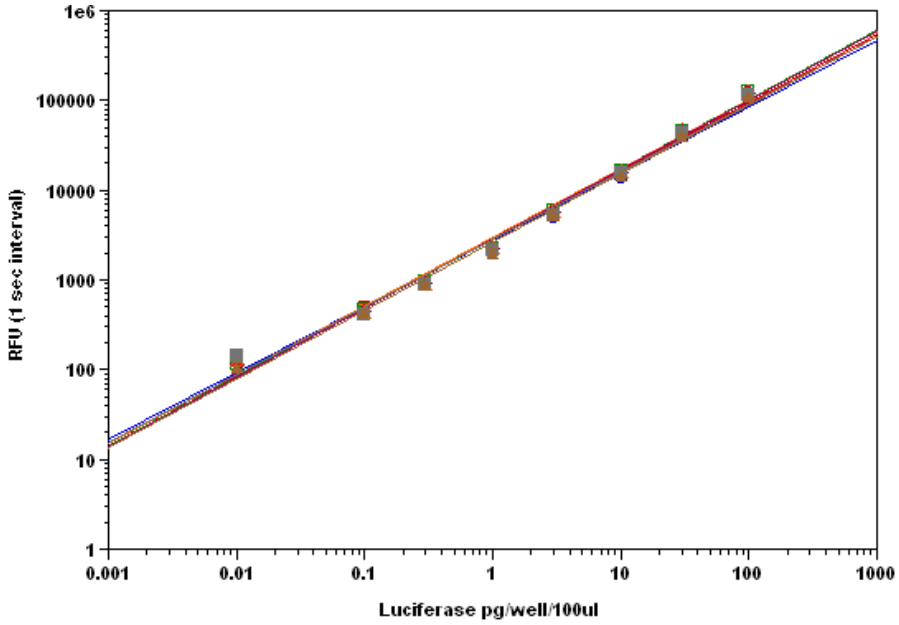

Figure 1. Luciferase dose response was measured with Amplite™ Luciferase Reporter Gene Assay Kit in a white 96-well plate with a NOVOstar plate reader (BMG Labtech). The kit can detect as low as 0.1pg/well luciferase with 20 minutes to 5 hours incubation without losing signal intensity. The integration time was 1 second. The half life is more than 4 hours.

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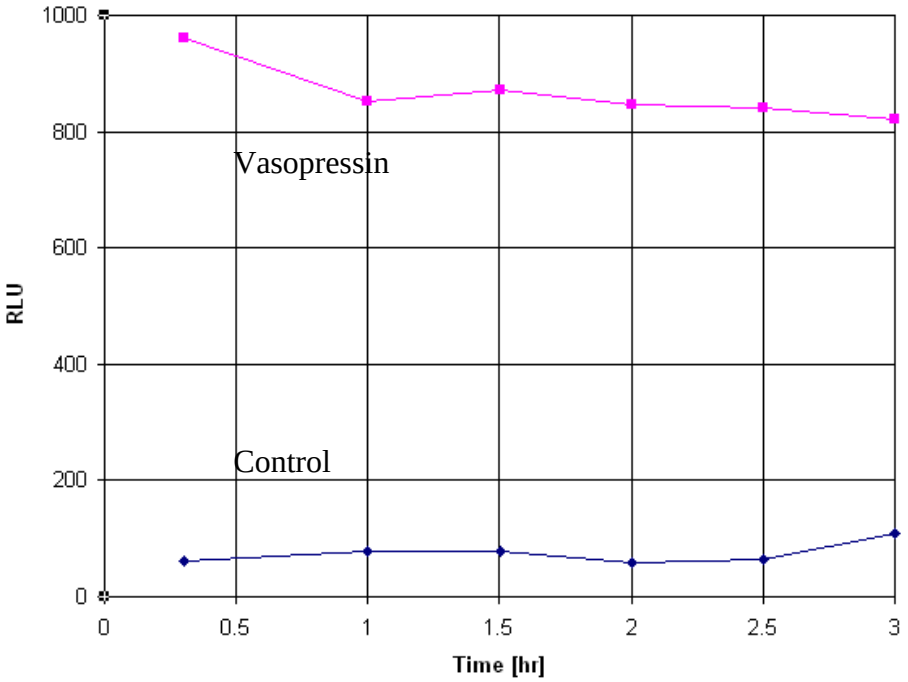

Figure 2. Reaction Kinetics of CHO-V_2R-Luc cells using Amplite™ Luciferase Reporter Gene Assay Kit. CHO cells stably transfected with pCRE-luciferase gene and human Vasopressin receptor 2 (V_2R) were plated into a Costar white wall/clear bottom 384-well plate at 15,000 cells/well/25 µL. Cells then were treated with 100 nM of vasopressin in a 37 ^oC , 5% CO_2 incubator for 4 hours. 25 µL of luciferase assay solution was added into the well. The kinetic data was taken every 30 minutes for up to 3 hours with a NOVOstar plate reader (BMG Labtech). The vasopressin induced luciferase signal is stable for more than 3 hours.

References

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