Amplite™ Luciferase Reporter Gene Assay Kit *Bright Glow*

Ordering Information:
Product Number: #12518 (1 plate); #12519 (10 plates)

Instrument Platform:
Luminescence microplate readers

Storage Conditions:
Keep in freezer and avoid light

Introduction

Common reporter genes include β-galactosidase, β -glucuronidase and luciferase. The advantages of a luciferase assay are the high sensitivity, the absence of luciferase activity inside most of the cell types, the wide dynamic range, rapidity 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 extremely high sensitivity, and can be used for the assays that require demanding sensitivity.

The kit provides a fast, simple, and homogeneous bioluminescence assay for studying gene regulation and function. This assay is based on the using 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 product 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 extremely 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 characteristic of the "glow-type" signal with a half-life of two to four hours providing a consistent signal across large batches of plates. It is compatible with the use of standard cell growth media.

$$\text{Luciferin} + \text{ATP} + O_2 \xrightarrow{Mg2+} \text{Oxyluciferin} + \text{AMP} + \text{Pyrophosphate} + CO2 + \text{light}\ (\sim 560\ \text{nm})$$

Kit Key Features

Sensitive:	Can detect as low as 0.1 pg luciferase/well.
Continuous:	Stable luminescence, suitable for manual or automated operations with no mixing or separations required.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

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Materials	#12518	#12519
	1 plate	10 plates
Component A: Luciferase Sensor (Light-sensitive)	1 vial	10 vials
Component C: Assay buffer	1 vial (10 ml)	1 bottle (100 ml)

Assay Protocol (for 1 plate)

Brief Summary

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

1. Prepare Cells (or Samples)

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

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/well (90 µL for 96-well) or 500-5,000 cells/well (20 µL for 384-well poly-D lysine plates). Centrifuge the plates at 800 rpm for 2 minutes with break off prior to the experiments.
Note1: 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.
Note2: For all luminescent experiments, it is recommended using white plates for achieving the best results.

2. Prepare Luciferase Assay Solution

2.1 Thaw 1 vial each of the components to room temperature before use.

2.2 Transfer 10 mL of component B (reaction buffer) into vial A, and mix well.
Note: aliquot and store the unused components B at -20°C, avoiding freeze/ thaw cycles.

3. Run Luciferase Assay

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

3.2 Incubate the cell plates in 37°C, 5% CO2 incubator for the desired period of time, typically 4 hours to overnight in a 5% CO_2, 37°C incubator.

3.3 Add 100 µL (96-well plate) or 25 µL (384-well plate) per well of luciferase assay solution (prepared in Step 2) for 10-20 min at room temperature, avoid light.

3.4 Read luminescence intensity using a standard 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 of 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 96-well plate, 25 µL for 384-well plate).
4.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of luciferase assay solution (prepared in step 2).
4.4 Incubate the reaction mixture for 10- 20 min at room temperature, avoid light.
4.5 Record the luminescence intensity using a standard luminometer.
4.6 Generate the ATP 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 can be varied depending upon the sources of the growth media or the microtiter plates. The typical data are included in Figures 1 (Luciferase titration curve).

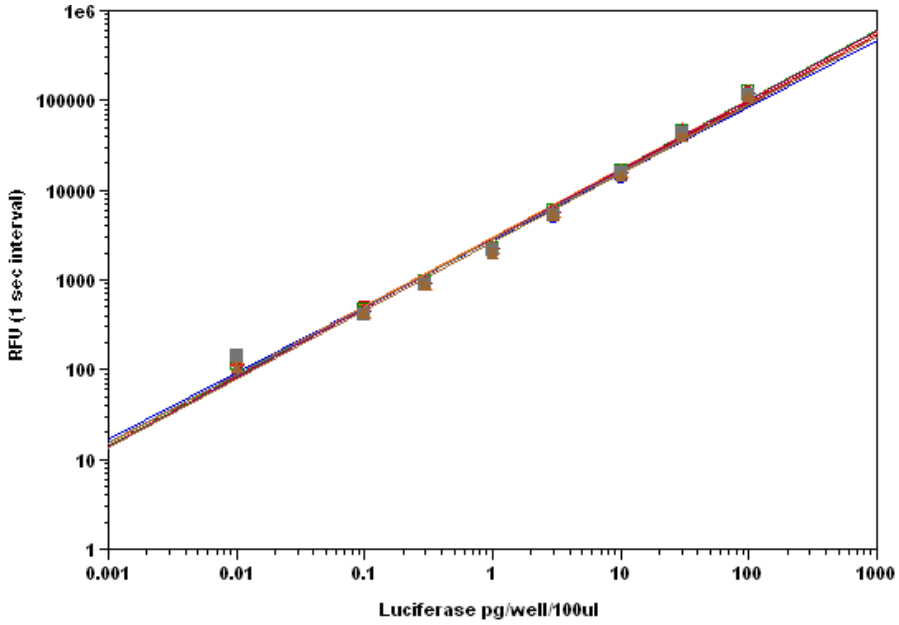

Figure 1. Luciferase dose response was measured with the Amplite™ Luciferase Reporter Gene Assay Kit on 96-well white plate using a NOVOstar plate reader (BMG Labtech). The kit can detect as low as 0.1pg/well luciferase between 20 min to 5 hr incubation time without losing signal intensity). The integration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

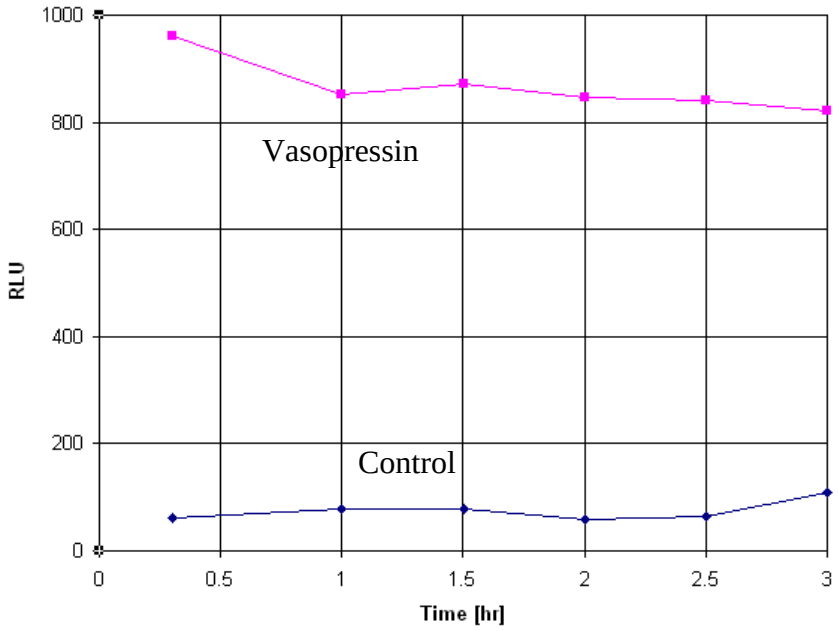

Figure 2. Reaction Kinetics of CHO-V_2R-Luc cells by using Amplite™ Luciferase Reporter Gene Assay Kit. CHO cells stably transfected with pCRE-luciferase gene and human Vasopressin receptor 2 (V2R) were plated into 384-well white wall/clear bottom Costar plate at 15,000 cells/well/25µl. Cells then were treated with 100 nM of vasopressin at 37°C, 5% CO_2 incubator for 4 hours. 25µl of Amplite™ Luciferase Reporter Gene Assay Kit luciferase assay reagent were added into the well. The kinetic data was taken every 30 minutes up to 3 hours using a NOVOstar plate reader (BMG LabTech). The vasopressin induced luciferase signal is stable for more than 3 hrs.

References:

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