

Amplite™ Gaussia Luciferase Reporter Gene Assay Kit

Bright Glow

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 12530 (1 plate); 12531 (10 plates); 12532 (100 plates)	Keep in freezer Avoid exposure to light	Luminescence microplate readers

Introduction

The most versatile reporter gene is the firefly luciferase. Recently there is steadily increasing use of other luciferases, such as Gaussia luciferase since these reporters are smaller and do not require the presence of ATP. The bioluminescent enzyme derived from the marine copepod Gaussia prince is efficiently secreted from mammalian cells upon expression. Gaussia luciferase is a 20kDa protein which catalyzes coelenterazine oxidation by oxygen to produce light. Our Amplite™ Gaussia Luciferase Reporter Gene Assay Kits use a proprietary luminogenic formulation to quantify luciferase activity in cell medium. The formulation generates a luminescent product that gives strong luminescence upon interaction with Gaussia luciferase. The kits provide all the essential components that are compatible with HTS liquid handling instruments. They have high sensitivity and can be performed in a convenient 96-well and 384-well microtiter-plate format. The “glow-type” signal with a half-life of one hour provides a consistent signal across large number of assay plates. The assay is compatible with standard cell growth media.



Kit Components

Components	Cat. # 12530 (1 plate)	Cat. # 12531 (10 plates)	Cat. # 12532 (100 plates)
Component A: Luciferase Substrate (Light-sensitive)	1 vial	1 vial	2 vials
Component B: Reaction Buffer	1 vial (50 µL)	1 vial (0.5 mL)	1 bottle (5 mL)
Component C: Assay Buffer	1 bottle (5 mL)	1 bottle (50 mL)	1 bottle (500 mL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare samples (50 µL/well/96-well plate or 12.5 µL/well/384-well plate) → Add 50 µL/well/96-well plate or 12.5 µL/well/384-well plate of Gaussia luciferase assay solution → Incubate at room temperature for 10-15 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 plates 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 Gaussia luciferase assay solution:

- 2.1 Make 100X Gaussia luciferase assay stock solution: Transfer 50 µL (for #12530), 0.5 mL (for #12531) and 2.5 mL (for #12532) of Reaction Buffer (Component B) into 1 vial of Luciferase Substrate (Component A), and mix them well.
Note: Store the unused 100X Gaussia Luciferase substrate stock solution at -20 °C, and keep from light.

2.2 Make Gaussia luciferase assay solution: Add one volume of 100X Gaussia Luciferase assay stock_solution (from Step 2.1) to 100 volumes of Assay Buffer (Component C).

Note: The reconstituted Gaussia luciferase assay solution is very sensitive to light, should be kept from light. And it is not stable, should be prepared fresh, kept on ice and used within 2 hours.

3. Run Gaussia 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 plates in a 37 °C, 5% CO₂ incubator for a desired period of time, typically 4 hours to overnight.
- 3.3 Run Gaussia Luciferase Assay: Pipette 50 μL /well/96-well plate or 12.5 μL /well/384-well plate of the serial diluted Gaussia luciferase or culture supernatant into a microtiter plate, and then mix with 50 μL /well/96-well plate or 12.5 μL /well/384-well plate of the Gaussia luciferase assay solution (from Step 2.2). Incubate the plate at room temperature for 10 to 15 minutes, kept from light.
- 3.4 Read luminescence intensity with a luminometer.

Data Analysis

The luminescence in blank wells with the growth medium is used as a control, and is subtracted from the values for the sample wells. The background luminescence of the blank wells varies depending on the sources of the growth media or the microtiter plates.

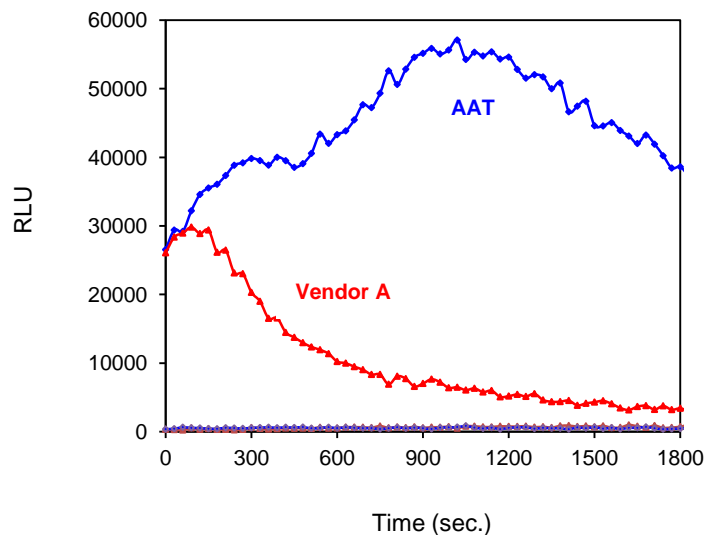


Figure 1. Secreted Gaussia Luciferase culture medium was measured with Amplite™ Gaussia Luciferase Reporter Gene Assay Kit (blue line) and a commercially available Gaussia Luciferase Assay Kit (red line) in a white 96-well plate with a NOVOstar plate reader (BMG Labtech).

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.