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

Common reporter genes include β-galactosidase, β-glucuronidase and luciferase. 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 Kit uses a proprietary luminogenic formulation to quantify luciferase activities in cell medium. Our formulation generates a luminescent product that gives strong luminescence upon interaction with *Gaussia* luciferase. The kit provides all the essential components that are compatible with HTS liquid handling instruments. The kit has 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.

Coelenterazine + O_2 → Coelenteramide + CO_2 + Light (~ 480 nm)

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 min → Read 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 (for 96-well plates) or 250-2,000 cells/20 µL/well (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/90 µL/well (for 96-well) or 500-5,000 cells/20 µL/well (for 384-well) poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with brake off prior to the experiment.
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 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 remaining 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 substrate 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 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-well 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 a 37 ^{o}C, 5% CO_2 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 min, 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.

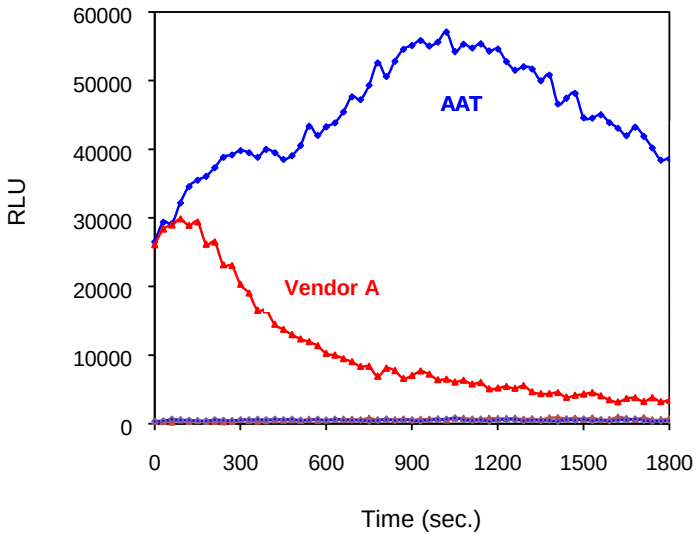

Figure 1. Sectreated Gaussia Luciferase culture medium was measured with Amplite ™ Gaussia Luciferase Reporter Gene Assay Kit (blue line) and a commercially available *Renilla* Assay Kit (red line) in a 96-well white plate with a NOVOstar plate reader (BMG Labtech).

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 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.

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