

Amplite™ Gaussia Luciferase Reporter Gene Assay Kit *Bright Glow*

Catalog number: 12530, 12531, 12532
Unit size: 1 plate, 10 Plates, 100 plates

| Component | Storage | Amount | | |
|---|---|-----------------|------------------|-------------------|
| | | Cat No. 12530 | Cat No. 12531 | Cat No. 12532 |
| Component A: Luciferase Substrate (Light-sensitive) | Freeze (<-15 °C), Minimize light exposure | 1 vial | 1 vial | 2 vials |
| Component B: Reaction Buffer | Refrigerate (2-8 °C), Minimize light exposure | 1 vial (50 µL) | 1 vial (0.5 mL) | 1 bottle (5 mL) |
| Component C: Assay Buffer | Freeze (<-15 °C) | 1 bottle (5 mL) | 1 bottle (50 mL) | 1 bottle (500 mL) |

OVERVIEW

Common reporter genes include beta-galactosidase, beta-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 activity 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 standard cell growth media.

AT A GLANCE

Protocol summary

1. Prepare samples (50 µL)
2. Add 50 µL Gaussia Luciferase working solution
3. Incubate at room temperature for 10 - 15 minutes
4. Monitor luminescence intensity

KEY PARAMETERS

Instrument: Luminescence microplate reader
Recommended plate: Solid white

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Gaussia Luciferase stock solution (100X):

Transfer 50 µL (for Cat#12530), 0.5 mL (for Cat#12531) or 2.5 mL (for Cat#12532) of Reaction Buffer (Component B) into 1 vial of Luciferase Substrate (Component A), and mix well.

PREPARATION OF WORKING SOLUTION

Dilute 100X Gaussia Luciferase assay stock solution using 1:100 dilution factor with Assay Buffer (Component C) to prepare Gaussia Luciferase working solution.

Note The reconstituted Gaussia luciferase working solution is very sensitive to light. It is not stable, should be prepared fresh, kept on ice, and used within 2 hours.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit

<https://www.aatbio.com/resources/guides/cell-sample-preparation.html>

SAMPLE EXPERIMENTAL PROTOCOL

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.
2. Incubate the cell plates in a 37°C, 5% CO₂ incubator for a desired period of time, typically 4 hours to overnight.
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 working solution.
4. Incubate the plate at room temperature for 10 to 15 minutes, protected from light.
5. Read luminescence intensity with a luminometer.

EXAMPLE DATA ANALYSIS AND FIGURES

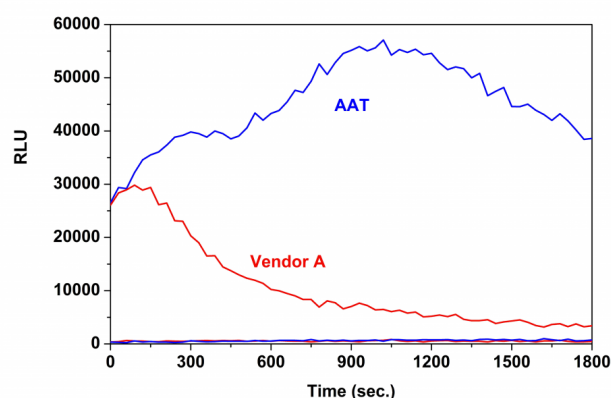


Figure 1. Secreted Gaussia Luciferase culture medium was measured with Amplite™ Gaussia Luciferase Reporter Gene Assay Kit (blue line) and a commercially available Gaussia Luciferase Assay Kit (red line) in a white 96-well plate with a NOVostar plate reader (BMG Labtech).

DISCLAIMER

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