

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

Catalog number: 12535, 12536, 12537 Unit size: 1 plate, 10 plates, 100 plates

| Component | Storage | Amount | | |
|--|---|------------------|-------------------|----------------------|
| | | Cat No. 12535 | Cat No. 12536 | Cat No. 12537 |
| Component A: 100X Luciferase Substrate (Light-Sensitive) | Freeze (<-15 °C), Minimize light exposure | 1 vial (100 μL) | 1 vial (1 mL) | 10 x 1 mL/vial |
| Component B: Assay Buffer | Freeze (<-15 °C) | 1 bottle (10 mL) | 1 bottle (100 mL) | 10 x100 mL/bottle |

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 Renilla luciferase since these reporters are smaller and do not require the presence of ATP. Our Amplite™ Renilla Luciferase Reporter Gene Assay Kit is designed to provide a fast and sensitive method to detect the luciferase from sea pansy (Renilla reniformis). It uses a proprietary luminogenic formulation to quantify Renilla luciferase activity in cell-based assays. Our formulation generates a luminescent product that gives strong luminescence upon interaction with Renilla luciferase. The kit provides all the essential components. It 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. This kit enables the measurement of primary expression or gene expression with wild type and the synthetic hRluc genes.

AT A GLANCE

Protocol summary

- 1. Prepare cell plates
- 2. Treat cells as needed3. Remove medium from cell plates
- Add Renilla Luciferase working solution (100 μL/well for 96-well plate or 25 μL/well for 384-well plate)
- 5. Incubate at room temperature for 5 10 minutes
- 6. Monitor luminescence intensity

Important Thaw all the kit components to room temperature before use. For all luminescent experiments, it is recommended to use white plates to get the best results.

KEY PARAMETERS

Instrument: Luminescence microplate reader

Recommended plate: Solid white

PREPARATION OF WORKING SOLUTION

 Add one volume of 100X Luciferase Substrate (Component A) to 100 volumes of Assay Buffer (Component B) to make Renilla Luciferase working solution.

Note The reconstituted Renilla Luciferase working solution is very sensitive to light, should be kept from light. In addition, 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.
- Incubate the cell plate in a 5% CO₂ incubator at 37°C for desired period of time, typically 4 hours to overnight.
- 3. Remove the medium completely.
- 4. Add 100 μ L (96-well plate) or 25 μ L (384-well plate) per well of Renilla Luciferase working solution.
- 5. Incubate the plate at room temperature for 5 10 minutes. Protect from light.
- 6. Monitor luminescence intensity with a luminometer.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RLU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Renilla Luciferase samples. We recommend using the Online Linear Regression Calculator which can be found at:

 $\frac{https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator}{}$

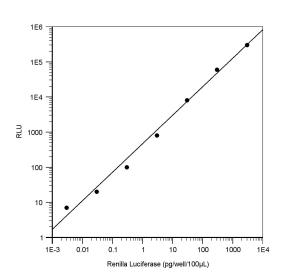


Figure 1. Renilla Luciferase was measured with Amplite™ Renilla Luciferase Reporter Gene Assay Kit in a white 96-well plate with a NOVOstar plate reader (BMG Labtech).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.