Amplite™ Renilla Luciferase Reporter Gene Assay Kit
Bright Glow

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Numbers: 12535 (1 plate) 12536 (10 plates); 12537 (100 plates)	Keep in freezer Avoid light	Luminescence microplate readers

Introduction

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.

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

Kit Components

Components	**12535 (1 plate)**	**12536 (10 plates)**	**12537 (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 (50µL/well/96-well plate or 12.5 µL/well/384-well plate)→ Add 50µL/well/96-well plate or 12.5 µL/well/384-well plate of Renilla luciferase assay solution → Incubate at room temperature for10-15 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 250-2,000cells/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 20,000-200,000 cells/90 µL/well for a 96-well poly-D lysine plate or 5000-50,000 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.
Note 3: It is highly recommend use phenol red free growth medium especially DMEM and MEM which is known to absorb the light emitted from luciferases and hence quench the signal observed.

Ordering:sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support:support@aatbio.com; Tel: 408-733-1055

2. Prepare Renilla luciferase assay solution:

2.1 Make 100X Renilla luciferase assay stock solution: Transfer 50 µL(for #12535), 0.5 mL (for #12536) and 2.5 mL (for #12537) of Reaction Buffer (Component B) into 1 vial of Luciferase Substrate(Component A), and mix them well.
Note: Store the unused 100X Renilla Luciferase substrate stock solution at -20 °C, and keep from light.

2.2 Make Renilla luciferase assay solution: Add one volume of 100X Renilla Luciferase assay stock solution (from Step 2.1) to 100 volumes of Assay Buffer (Component C).
Note: The reconstituted Renilla luciferase assay 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.

3. Run Renilla 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.

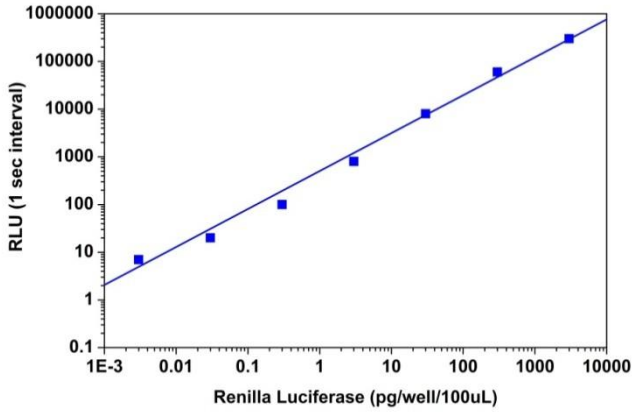
3.2 Incubate the cell plates in a 37 ^{o}C, 5% CO_2 incubator for desired period of time, typically 4 hours to overnight.

3.3 Add 100 µL (96-well plate) or 25 µL (384-well plate) per well of Renilla luciferase assay solution (from Step 2.2) and incubate the plate at room temperature for 10-15 minutes. Keep it from light.
Optional: Aspirate the medium off before adding Renilla luciferase assay solution.

3.4 Monitor luminescence intensity with a luminometer.

Data Analysis

The luminescence in blank wells with the growth medium is used as a control, and 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.



F**igure 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).

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AATBioquest. 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.