Amplite™ Luciferase Reporter Gene Assay Kit *Bright Glow*

Catalog number: 12518, 12519, 12520
Unit size: 1 plate, 10 plates, 100 plates

Component	Storage	Amount		
		Cat No. 12518	Cat No. 12519	Cat No. 12520
Component A: Luciferase Sensor (Light-sensitive)	Freeze (<-15 °C), Minimize light exposure	1 bottle	1 bottle	1 bottle
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)	1 bottle (100 mL)	1 bottle (1000 mL)

OVERVIEW

Common reporter genes include beta-galactosidase, beta-glucuronidase and luciferase. The advantages of a luciferase assay are the high sensitivity, the absence of luciferase activity inside most of the cell types, the wide dynamic range, rapidity and low cost. The most versatile and common reporter gene is the luciferase of the North American firefly Photinus pyralis. The protein requires no posttranslational modification for enzyme activity. It is not even toxic in high concentration (in vivo) and can be used in pro- and eukaryotic cells. The firefly luciferase catalyzes the bioluminescent oxidation of luciferin in the presence of ATP, magnesium and oxygen. This Amplite™ Luciferase Reporter Gene Assay Kit uses a proprietary luminogenic formulation to quantify luciferase activity in live cells and cell extracts. Our formulation generates a luminescent product that gives strong luminescence upon interaction with luciferase. The kit provides all the essential components with our optimized 'mix and read' assay protocol that is compatible with HTS liquid handling instruments. It has extremely high sensitivity, and can be used for the assays that require demanding sensitivity.

AT A GLANCE

Protocol summary

1. Prepare cells (samples) with test compounds (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)
2. Add equal volume of Luciferase Sensor working solution
3. Incubate at room temperature for 10 - 20 minutes
4. Monitor luminescence intensity at 560 nm

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

1. For **Cat.# 12518**, transfer the whole content of Reaction Buffer (Component B) into the bottle of Luciferase Sensor (Component A) and mix well to make Luciferase Sensor working solution.

2. For **Cat.# 12519**, add 10 mL of Reaction Buffer (Component B) and for **Cat.# 12520**, add 100 mL of Reaction Buffer (Component B) into the bottle of Luciferase Sensor (Component A) and mix well. Then, transfer the resulted solution back to the bottle of Reaction Buffer (Component B). Multiple washes are necessary to completely transfer the contents.

Note The reconstituted Luciferase Sensor working solution is not stable. Protect from light.

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

Run Luciferase assay:

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 plate in a 5% CO_2 incubator at 37°C for desired period of time, typically 4 hours to overnight.

3. Add 100 µL (96-well plate) or 25 µL (384-well plate) per well of Luciferase Sensor working solution.

4. Incubate the plate at room temperature for 10 - 20 minutes. Keep from light.

5. Monitor luminescence intensity with a luminometer.

Establish standard Luciferase calibration curve:

Note Luciferase standard curve should be generated together with the above assay if the absolute amount of Luciferase in samples needs to be calculated.

1. Make a series dilutions of Luciferase in PBS buffer with 0.1% BSA by including a sample without Luciferase (as a control) for measuring background luminescence.

   Note Typically Luciferase concentrations from 1 pg/mL to 1 ng/mL are appropriate.

2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of diluted Luciferase solution into an empty plate.

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Luciferase working solution.

4. Incubate the reaction mixture at room temperature for 10 - 20 minutes, protected from light.

5. Record the luminescence intensity with a standard luminometer.

6. Generate the Luciferase standard curve.

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 base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Luciferase samples. We recommend using the Online Linear Regression Calculator which can be found at:

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

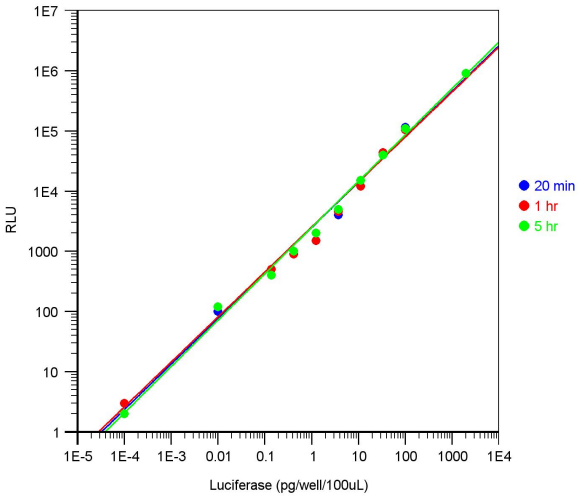

Figure 1. Luciferase dose response was measured with Amplite™ Luciferase Reporter Gene Assay Kit in a white 96-well plate with a NOVOstar plate reader (BMG Labtech). The kit can detect as low as 0.1pg/well luciferase with 20 minutes to 5 hours incubation without losing signal intensity. The integration time was 1 second. The half life is more than 4 hours.

DISCLAIMER