



TECHNICAL MANUAL

Firefly Luciferase Reporter Gene Luminescence Assay Kit

- **SKU CODE:** MAES0318
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Intended use

This kit can be used to detect high expression levels of firefly luciferase.

2. Detection principle

Firefly luciferase reporter gene detection is a reporting system that uses luciferin as a substrate to detect the activity of firefly luciferase.

The detection principle of this kit: In the presence of oxygen, ATP, and magnesium ions simultaneously, firefly luciferin is catalyzed by firefly luciferase in the sample to oxidize luciferin and emit yellow-green light. The expression of firefly luciferase in the sample can be detected by the chemiluminescence instrument.

Compared with MAES0315, the luminescence performance of this kit is longer and more stable, making it suitable for detection of systems with higher expression levels and diversity.

3. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	7 mL x 1 vial	14 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 2	Substrate	Powder x 2 vials	Powder x 4 vials	-20 °C, 12 months, shading light
	Black Clear-bottom Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

4. Materials prepared by users

Instruments:

Chemiluminescence immunoassay analyzer or multifunctional microplate reader (with the function of detecting luminescence)

5. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of working solution:** Dissolve one vial of substrate with 3 mL of buffer solution, mix well to dissolve. Aliquot and store at -20 °C for 1 month, protected from light.

6. Sample preparation

Cell samples:

The cells were inoculated and designed according to the following groups:

Blank group: The cells without transfection treatment;

Control group: The cells were transfected with plasmids without drug stimulation.

Experiment group: The cells were transfected with plasmids and stimulated with drugs according to experimental design.

7. Key points of the assay

The working solution should be aliquoted and stored at -20 °C. Avoid repeated freeze/thaw cycles.

8. Operating steps

Add 100 µL of working solution into black clear-bottom microplate, and mix fully with chemiluminescence immunoassay analyzer. Incubate for 25 min. Measure the luminescence values of each well. (The amount of working solution added to cells cultured in 24-well or other plate holes can be referred to the following table. After mixing well, aspirate 100 µL of working solution and add it to the black clear-bottom microplate for detection)

Cell culture plate: 96-well plate - Working solution: 100 µL

Cell culture plate: 24-well plate - Working solution: 200 µL

Cell culture plate: 12-well plate - Working solution: 300 µL

Cell culture plate: 6-well plate - Working solution: 500 µL

9. Calculation

Experiment group = $F_1 - F_3$

Control group = $F_2 - F_3$

[Note]

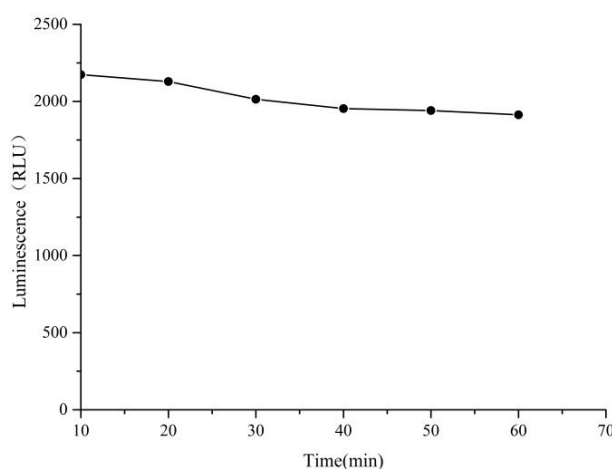
F_1 : The luminescence values of experiment group.

F_2 : The luminescence values of control group.

F_3 : The luminescence values of blank group.

10. Appendix I Performance Characteristics

Firefly luciferase reaction curve:



11. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.