TECHNICAL MANUAL

Firefly Luciferase Reporter Gene Luminescence Assay Kit

- **SKU CODE:** MAES0315
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit can be used to detect low 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, firefly luciferin in the sample is catalyzed by firefly luciferase to oxidize luciferin and emit yellow-green light. The expression of firefly luciferase in the sample can be detected using a chemiluminescence instrument.

3. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Lysis Buffer	25 mL x 1 vial	50 mL x 1 vial	-20 °C, 12 months
Reagent 2	Buffer Solution	7 mL x 1 vial	14 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 3	Substrate	Powder x 2 vials	Powder x 4 vials	-20 °C, 12 months, shading light
	Black Clear-bottom Culture Plate	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. **The 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:

1. 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

2. Cell lysis:

- Adherent cells: According to the table below, add the corresponding volume of lysis buffer. Place on ice and mix well every 5 min, lyse for 10 min.

- Suspension cells: Centrifuge at 500 x g for 3 minutes at 4 °C to remove insoluble material. According to the table below, add the corresponding volume of lysis buffer. Place on ice and mix well every 5 min, lyse for 10 min. Centrifuge at 10,000 x g for 10 minutes at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.

Cell culture plate | 96-well plate | 24-well plate | 12-well plate | 6-well plate

Lysis buffer (μL) | 100 | 200 | 300 | 500

7. The key points of the assay

1. The working solution should be aliquoted and stored at -20 °C, and repeated freeze/thaw cycles should be avoided.
2. It is recommended that the number of samples for an experiment be controlled within 10 samples.

8. Operating steps

1. Sample well: add 20 μL of samples into the corresponding well.
2. Add 20 μL of working solution into sample wells, and mix fully with chemiluminescence immunoassay analyzer for 2-3 s. Measure the luminescence values of each well.

9. Calculation

Experiment group = F_1 - F_3

Control group = F_2 - F_3

[Note]

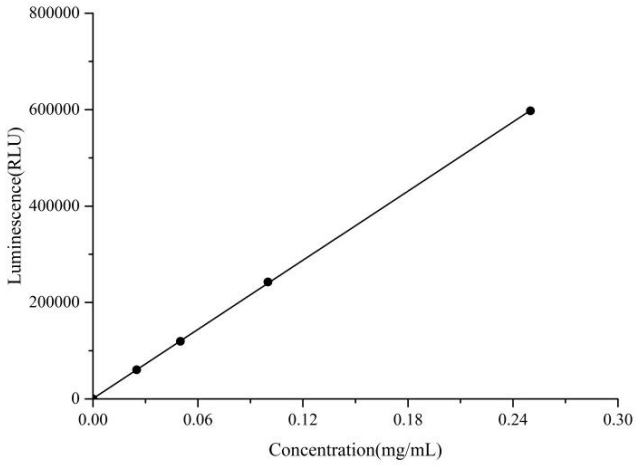
F_1: The luminescence values of experiment group.

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:



Concentration (mg/mL) | 0 | 0.025 | 0.05 | 0.1 | 0.25

Luminescence value | 67 | 61286 | 114251 | 231454 | 563113

| 89 | 59434 | 124583 | 253566 | 632215

Average luminescence value | 78 | 60360 | 119417 | 242510 | 597664

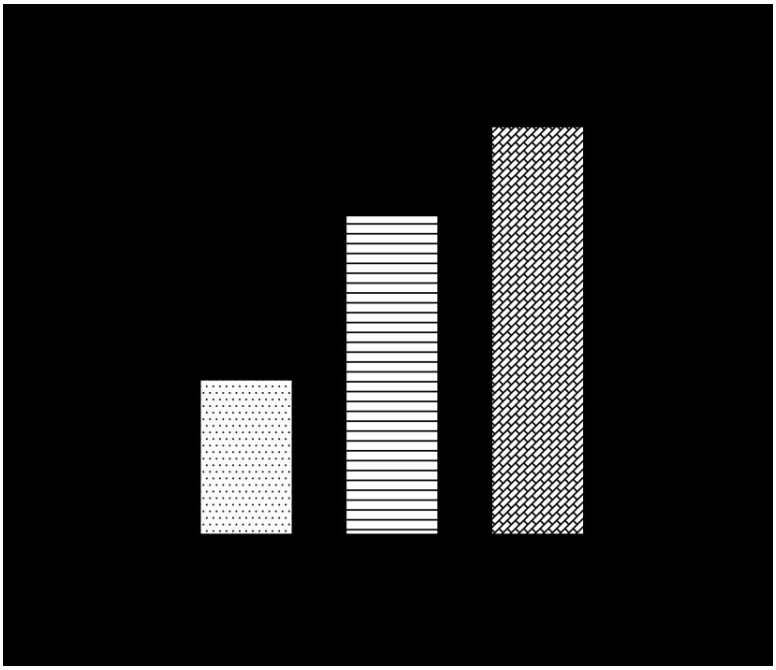
Absolute luminescence value | 0 | 60282 | 119339 | 242432 | 597586

11. Appendix II Example Analysis

Example analysis:

To detect the effect of tumor necrosis factor (TNF-α) induced stimulation on the expression of firefly luciferase NF-κB response element plasmids:

293T cells were inoculated in 24-well plates, approximately 5×10^4 cells per well, and cultured overnight. Transfection reagent containing pNF-κB-luc plasmid was added, and the cells were cultured for 24 h after observation of good state. Different concentrations of TNF-α were added to stimulate induction for 5 h. Following the instructions to measure the chemiluminescence value, the result is as follows:



12. 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.