（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F075

Specification：48T/96T

Measuring instrument：Chemiluminescence immunoassay analyzer

Elabscience® Firefly Luciferase Reporter Gene Luminescence Assay Kit (Glow Type)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Intended use

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

Detection principle

Firefly Luciferin 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 at the same time, firefly luciferin is catalyzed by firefly luciferase in the sample to oxidize luciferin and emit yellow-green light, and the expression of firefly luciferin in the sample can be detected by the chemiluminescence instrument. Compared with E-BC-F072, the luminescence performance of this kit is longer and more stable, and it is suitable for the detection of this system with higher expression levels and diversity.

Kit components & storage

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

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of working solution:

Dissolve one vial of substrate with 3 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20 ℃ for 1 month protected from light.

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.

The key points of the assay

The working solution should be aliquoted storage at -20 ℃, and avoid repeated freeze/thaw cycles is advised.

Operating steps

Add 100 μL of working solution into black clear-bottom microplate, and mix fully with chemiluminescence immunoassay analyzer. Incubated 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, absorb 100 μL of working solution and add it to the black clear-bottom microplate for detection)

Cell culture plate	96-well plate	24-well plate	12-well plate	6-well plate
Working solution (μL)	100	200	300	500

Calculation

$$\text{Experiment group} = F_1 - F_3$$

$$\text{Control group} = F_2 - F_3$$

[Note]

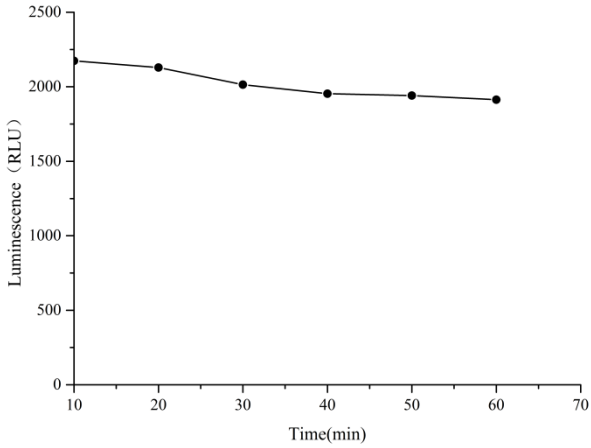
F_1: The luminescence values of experiment group.

F_2: The luminescence values of control group.

F_3: The luminescence values of blank group.

Appendix I Performance Characteristics

Firefly luciferase reaction curve of:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.