Quantitatively measure the expression of firefly luciferase

MBI Evolution LUCY Luciferase Assay Kit
Cat #MBILUCY

RUO Research Use Only REF 21200 Σ 100 -65 ℃ -85℃

DESCRIPTION

- MBI Evolution LUCY Luciferase Assay Kit is designed for cell biology to study gene expression and other cellular events related to gene expression.
- Gene expression is generally studied by linking a promoter sequence to an easily detectable "reporter" gene such as that encoding for firefly luciferase.
- Firefly luciferase catalyzes the oxidative carboxylation of luciferin, reacting with the highest efficiency of any known bioluminescence reaction.
- The assay is very sensitive because its light production has the highest quantum efficiency known for any chemiluminescent reaction, and no background luminescence is found in the host cells or the assay chemistry.
- The assay is rapid, requiring only a few seconds per sample.

INTRODUCTION

The Luciferase Assay is substantially improved over conventional assay methods in both sensitivity and simplicity. Light is produced by converting the chemical energy of luciferin oxidation through an electron transition, forming the product molecule oxyluciferin. Firefly luciferase is a 62kDa protein which is active as a monomer and does not require subsequent processing for its activity. However, several factors may affect the sensitivity and success of the assay including pH, temperature, and substrate concentration. To ensure maximum sensitivity, the assay is performed in the presence of excess ATP, luciferin and Mg^{2+} in a buffer that will maintain a pH of 7.8. In the conventional assay for luciferase, a flash of light is generated that decays rapidly after the enzyme and substrates are combined. Generally, 100-fold greater sensitivity can be achieved over the chloramphenicol acetyltransferase (CAT) assay. The Luciferase Assay System was developed for reporter quantitation in mammalian cells.

STORAGE AND STABILITY

- Storage condition : Store the products at each temperature ; 5X Cell Lysis Buffer **(4 ºC)** and Luciferase assay Buffer **(4 ºC)**, Luciferin Solution **(-60 ºC or below)**.
- Expiration date : Luciferin Solution is stable for 12 months at -60 ºC or below, for 1 months at -15 to -25 ºC if stored in aliquots, or for 1 week at 2 to 8 ºC. Avoid repeated freezing and thawing. Luciferase Assay Buffer and 5X Cell Lysis Buffer can be stored for up to 24 months without showing any reduction in performance and quality under appropriate storage condition. The expiration date is labeled on the product label.
- Store protected from light, as Luciferin Solution is oxidized when exposed to light.

KIT CONTENTS

Label	Contain
5X Cell Lysis Buffer	15 ml x 2 bottle (30ml)
Luciferase Assay Buffer	9 ml x 2 bottle (18ml)
Luciferin Solution	1 bottle (4.5 ml)

ADDITIONAL REQUIRED EQUIPMENT

- Centrifuge (Swing rotor)
- Pipettes and pipette tips (aerosol resistant)
- Centrifuge tube
- Automatic or Manual Luminometers
- White flat bottom microplate (96 well) or luminometer tube.

NOTICE BEFORE USE

MBI Evolution LUCY Luciferase Assay Kit is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. MBI Evolution LUCY Luciferase Assay Kit is developed, designed, and sold for research purpose only. It is not intended to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals.

APPLICATIONS

MBI Evolution LUCY Luciferase Assay Kit with high sensitivity can be used in manual or automated luminometers, in microplate or tube format as well as in scintillation counters or with photographic films. In order to achieve maximum sensitivity the use of luminometers operating with ultra-fast photon counters is recommended.

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PROTOCOL

Equilibrate all reagents (Avoid multiple freeze-thaw cycles) to room temperature before starting the assay.

This protocol is optimized for use with eukaryotic cell cultures.

1. Prepare an proper amount of 1X Cell Lysis Buffer by diluting 5X Cell Lysis Buffer into distilled water.

   NOTE : The following protocol is designed for use with adherent cultures growing in 6 well culture plates. If you are using plates, wells, or flasks of a different size, adjust the volume proportionally.

2. Remove media from cell culture plates and rinse twice with phosphate buffered saline (PBS without Ca^{2+} and Mg^{2+}).
3. Add 100 µl 1X Cell Lysis Buffer to cells and shake at room temperature for 15–20 min. Alternatively, cells may be lysed at 4°C to minimize protease activity. If performing lysis at 4°C, allow cell lysate to reach room temperature before continuing with protocol.
4. Harvest cells by scraping or pipetting and transfer to a 1.5-ml microcentrifuge tube.

NOTE : Spin cells at 13,000 rpm at room temperature for 30sec - 1min to remove cellular debris.

NOTE : Samples should be assayed within 20 min. For measurements that require longer time points or for assays that are to be completed at a later date, extracts may be stored for up to one month at –70°C.

Plate or Flask Size	Surface Area (mm^2)	Relative Area*	Recommended Volume of 1X Cell Lysis Buffer
96 well	32	0.03 X	10 µl
24 well	200	0.21 X	30 µl
12 well	401	0.42 X	50 µl
6 well	962	1 X	100 µl
35 mm	962	1 X	100 µl
60 mm	2,827	2.9 X	250 µl
100 mm	7,854	8.2 X	500 µl
150 mm	17,671	18.4 X	1.2 ml
T-25	2,500	2.6 X	250 µl
T-75	7,500	7.8 X	500 µl
T-160	16,000	16.6 X	1.0 ml

* Relative area is expressed as a factor of the surface area of a 6 well culture plate.

6. Prepare mix solution to a well containing 160µl Luciferase Assay Buffer and 40µl Luciferin Solution(pre-equilibrated to room temperature) in a 96 well cell culture plate or a luminometer tube.
7. Before starting the chemiliminescence reaction, transfer the 96 well cell culture plate into an appropriate luminometer.
8. Start the reaction by adding 50ul prepared cell lysate.
9. Measure light emission for 10 sec after adding prepared cell lysate.

NOTE : After an almost constant light emission over a period of 30s, the light production decreases with a half-life about 5 min.

TROUBLE SHOOTING GUIDE

This troubleshooting guide may be helpful in solving problems that may frequently arise. The Support Team at MBI are always happy to answer any questions you may have about the information or protocol in this manual or other molecular biology applications.

Problem / Possible cause	Recommendation
Intra-assay Variability	
1) Pipetting error	• Use larger sample volumes to minimize variability caused by pipetting error.
2) Temperature changes	• Be sure all reagents have reached room temperature before performing assay.
3) Allowing sample and buffer to sit for extended periods of time.	• Work quickly to minimize the time between adding and initiating the reaction.
4) Reagent degratation	• Store all reagents at each temperature.
Abnormally Low Light from Assay	
1) Improper pH	• Test pH of each reagent and adjust to 7.8 if necessary.
2) Improper substrate concentrations	• Check that the correct volume of each reagent is being added to the assay reaction and adjust if necessary.
3) Presence of interfering substances	• Be sure to wash cells thoroughly with PBS 2–3 times before performing lysis.
High Background	
1) Contaminated reagents	• Reagents may become contaminated by carry-over from pipette tips. Be sure to change tips between reaction components and/or samples. Replace component if necessary.
2) Contaminated injector lines	• Flush injector lines throughly with distilled water.

MBI Lab Equipment

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