Firefly Luciferase Assay Kit

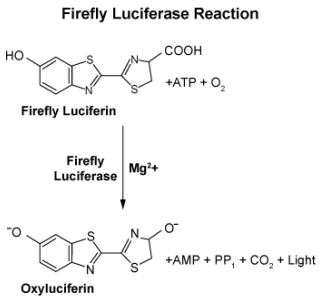
Protocol for MIR 5950

Mirus The Transfection Experts

Quick Reference Protocol, MSDS and Certificate of Analysis available at mirusbio.com/5950

INTRODUCTION

Firefly luciferase from *Photinus pyralis*, a 61 kDa monomer, can be utilized as a reporter gene assay for many biotechnology applications including monitoring transient luciferase expression levels in mammalian cells. Maximum light output is achieved when substrate and co-factors are present in large excess, resulting in a direct correlation between the light emitted from the reaction and the number of luciferase enzyme molecules present. Luciferase is not endogenously expressed in mammalian cells, making the luciferase assay an ideal genetic reporter system with a very high signal:background ratio. Firefly luciferase is a cytoplasmic protein that must be released from cells through lysis prior to quantification.



The Mirus Bio Firefly Luciferase Assay Kit is a rapid and quantitative detection assay with high sensitivity across a broad dynamic range. Since the light is emitted briefly, this kit it is only compatible with luminometers equipped with injectors. For ease of use, reaction buffer and substrate are combined in a single tube formulation, Substrate Solution F.

SPECIFICATIONS

Storage	Store Substrate Solution F and Luciferase Cell Lysis Solution at –20°C. ***Before each use***, warm to room temperature and vortex gently.
Product Guarantee	6 months from the date of purchase, when properly stored and handled.

CAUTION: Standard safe laboratory practices should be maintained when using all solutions. ***Please refer to product MSDS for full safety precautions.***

MATERIALS

Materials supplied

Firefly Luciferase Assay Kit (MIR 5950) is supplied in the following format. Please inquire about a custom quote for bulk quantities.

Product No.	Component	Volume
MIR 5950	Substrate Solution F	1 × 10 ml
	Luciferase Cell Lysis Solution	5 × 12 ml

Materials required, but not supplied

- Luminometer (injection capable)
- 96-well opaque microplates or compatible tubes (depending on luminometer set-up)
- Micropipettes
- Serological pipet
- PipetAid

PROTOCOL

A. Cell Harvest and Lysis

(Typically 24-48 hours post-transfection)

1. Thaw Substrate Solution F and Luciferase Cell Lysis Solution for ≤10 minutes in a 37°C water bath. Remove the solution immediately after thawing. Each vial can undergo up to 8 freeze-thaw cycles without loss of activity.
2. After thawing is complete, gently vortex Substrate Solution F. Ensure clarity of the solution prior to use by pulling up the thawed solution in a 10 ml serological pipet.
3. Remove the plates containing cells from the incubator.
4. Gently aspirate the entire volume of medium from each well. When using a vacuum pump aspirator, the addition of a micropipet tip to the end of a glass Pasteur pipet reduces the removal rate, thereby decreasing the risk of disturbing cells.
   NOTE: To remove media from suspension cells, centrifuge for 5 minutes at 300 x g. Alternatively, cells can be lysed in most media formulations by adding an equal volume of Luciferase Cell Lysis Solution to the cell-containing media.
5. Add 500 µl of Luciferase Cell Lysis Solution per well of a 6-well plate. Please refer to **Table 1** below for the appropriate volume for alternate plate formats.
6. Incubate on a rocking platform at room temperature for a minimum of 10 minutes. Cell lysis can be verified using a light microscope.

To avoid loss in activity, promptly remove Substrate Solution F and Luciferase Cell Lysis Solution from the 37°C water bath after thawing.

Gently vortex Substrate Solution F to resuspend any precipitate that may have formed during the thawing process.

For adherent cells, completely remove all of the media from the well prior to adding the Luciferase Cell Lysis Solution.

Table 1. Recommended Luciferase Cell Lysis Buffer Volumes

Culture vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate
Surface area	0.35 cm^2	1.0 cm^2	1.9 cm^2	3.8 cm^2	9.6 cm^2
Luciferase Cell Lysis Buffer	50 µl	75 µl	125 µl	250 µl	500 µl

B. Luciferase Assay

1. After the lysis incubation period, transfer 10 µl of cell lysate from each well into an opaque 96-well assay plate (or into a tube for non-microplate luminometers).
2. Measure luminescence with a luminometer using the following settings:
   a. 100 µl injection (Substrate Solution F)
   b. 0.5 second delay before measurement reading
   c. 10 second integration time

RELATED PRODUCTS

- *Trans*IT-X2® Dynamic Delivery System
- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits
- *Trans*IT-siQUEST® Transfection Reagent
- *Trans*IT-TKO® Transfection Reagent
- Ingenio® Electroporation Solution and Kits
- *Label* IT® Plasmid Delivery Controls
- *Label* IT® RNAi Delivery Controls
- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits
- MiraCLEAN® Endotoxin Removal Kits

For details on the above mentioned products, visit www.mirusbio.com

Reagent Agent®

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at:
www.mirusbio.com/ra

Contact Mirus Bio for additional information.

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