INSTRUCTIONS

Pierce Firefly Luc One-Step Glow Assay Kit

16196 16197

2547.1

Number	Description
16196	**Pierce Firefly Luc One-Step Glow Assay Kit,** sufficient reagents to perform 100 assays for firefly luciferase activity in cultured mammalian cells **Kit Contents:** **Firefly Luc One-Step Substrate, Lyophilized (makes 100µL of 100X),** store at -20°C **Firefly Luc One-Step Assay Buffer,** 11mL, store at -20°C
16197	**Pierce Firefly Luc One-Step Glow Assay Kit,** sufficient reagents to perform 1000 assays for firefly luciferase activity in cultured mammalian cells **Kit Contents:** **Firefly Luc One-Step Substrate, Lyophilized (makes 1mL of 100X),** store at -20°C **Firefly Luc One-Step Assay Buffer,** 115mL, store at -20°C

Storage: Upon receipt store kit at -20°C. Kit is shipped on dry ice.

Table of Contents

Introduction

The Thermo Scientific™ Pierce™ Firefly One-Step Luc Glow Assay Kit contains reagents for assaying firefly luciferase activity in mammalian cell culture. The kit is recommended for luminometers without injectors or for high-throughput screening (HTS) applications. The convenient one-step, homogenous protocol minimizes handling steps supporting the use of automation. When used with Thermo Scientific™ Firefly Luc Plasmids (see Related Thermo Scientific Products), the kit provides a highly sensitive bioluminescent reporter assay system for the detection of promoter or pathway activity.

Firefly luciferase is a ~62kDa protein produced in nature by several species of the *Lampyridae* family of beetles, which includes the genera *Photinus* and *Luciola*. The Thermo Scientific™ Red Firefly Luciferase has an emission range of 560-700nm (λmax = 613 nm). Bioluminescent signal from firefly luciferase originates from the oxidation of D-Luciferin (Figure 1). Light output captured using a luminometer can be correlated with the amount of firefly luciferase protein produced and used to determine the activity of the promoter driving firefly luciferase expression. Firefly luciferase

bioluminescence provides researchers with a non-radioactive assay for measuring transcriptional activity of regulatory elements. Firefly luciferase reporter activity can be used to determine promoter activity, to study upstream pathway activity in the cell, or to determine effect of activators or inhibitors.

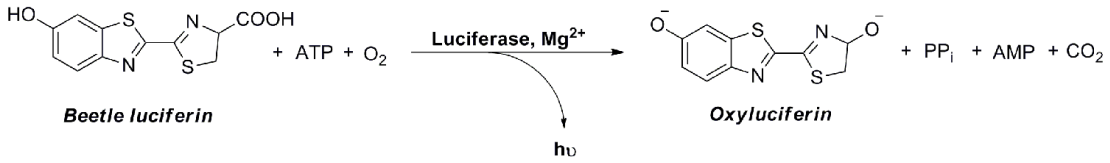
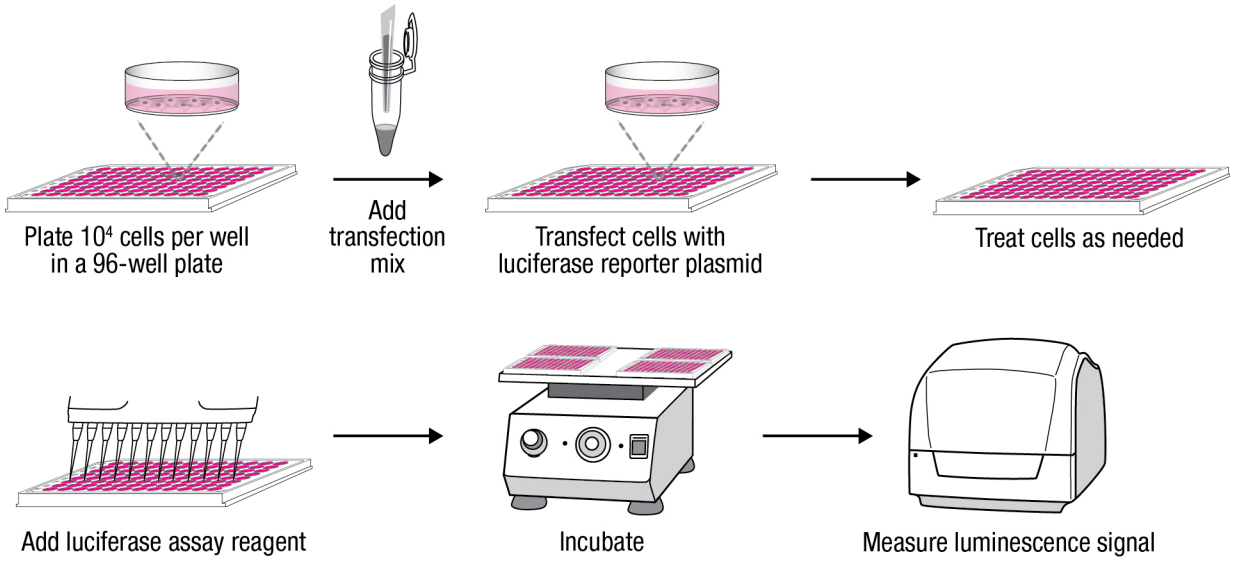


Figure 1. Firefly luciferase reaction. D-Luciferin substrate is oxidized by firefly luciferase in the presence of ATP and Mg^{2+}, producing firefly oxyluciferin, CO_2 and light.

Procedure Summary



Important Product Information

- For long-term use, store Firefly Luc One-Step Substrate **(**lyophilized) at -20°C protected from light.
- Once prepared, aliquot and store Firefly Luc One-Step Glow Assay Working Solution at -80°C for up to two months protected from light.
- Firefly Luc One-Step Glow Assay Working Solution must be at room temperature (20-25°C) before use and is stable for at least four hours at room temperature.
- To avoid cross-contamination, use a new disposable pipette tip for each transfer. If using a multi-channel pipette, always use a new disposable reagent reservoir.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Do not mix reagents from different lots. Discard unused working solutions after assay completion. Do not combine leftover reagents.

- Individual components may contain corrosives and/or preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Follow proper disposal procedures.
- Dispense and equilibrate to room temperature only the reagent volumes needed for the number of plates being used.

Additional Materials Required

- Reagents and equipment necessary to propagate mammalian cells in culture
- Reagents (e.g., Thermo Scientific™ TurboFect™ *in vitro* Transfection Reagent, Product No. R0533) and equipment necessary to transfect plasmid DNA into mammalian cells
- Laboratory platform shaker
- Pipettes and/or liquid handling equipment
- Luminometer or other luminescence-monitoring instrument

  Note: Reagent injectors are not necessary for this assay.
- White or black, opaque 96- or 384-well microplates

Material Preparation

100X Firefly Luc One-Step Substrate Solution	Reconstitute lyophilized Firefly Luc One-Step Substrate pellet in 100µL (100-assay kit) or 1mL (1000-assay kit) of ultrapure water. Store at -80°C for up to two months.
Firefly Luc One-Step Glow Assay Working Solution	Dilute 100X Firefly Luc One-Step Substrate Solution 1:100 in Firefly Luc One-Step Assay Buffer and mix well. Store at -80°C for up to two months. Example: For 100 reactions, add 100µL of 100X Firefly Luc One-Step Substrate Solution to 10mL of Firefly Luc One-Step Assay Buffer. Use 100µL per reaction. Prepare sufficient reagent to allow for dispensing errors.

Procedure for Firefly Luciferase Glow Assay

A. Cell Transfection

Note: Plate enough wells to perform the experiment in triplicate and include appropriate controls (e.g., non-transfected cell control and non-treated cell control) in each experiment.

Note: Use of a visual transfection control is highly recommended. For example, transfect a separate well with a constitutively expressed GFP plasmid and observe GFP expression using a fluorescence microscope.

1. Plate ~10,000 cells/well in a 96-well plate using 100µL of culture media per well. If using a different size plate, adjust the cell number accordingly. Incubate plates overnight at 37°C in 5% CO_2. Use only cells growing in log phase at a passage number ≤ 15.
2. Use a standard protocol to transfect mammalian cells with a firefly luciferase plasmid construct containing a constitutive (i.e., CMV, TK) or inducible promoter.
3. Incubate cells at 37°C in 5% CO_2 in a cell culture incubator.
4. Proceed with individual experimental protocol for cell treatment.

B. Firefly Luc One-Step Glow Assay

1. Remove the 96-well cell culture plate from the incubator.
2. Allow the plate to equilibrate to room temperature for 15 minutes.
3. Add 100µL of Firefly Luc One-Step Glow Assay Working Solution to each well.
4. Shake the plate on a plate shaker at medium speed for 3 minutes.

5. Incubate the plate at room temperature for ≥ 10 minutes.
6. Program the luminometer according to the manufacturer's recommendations.

   Note: Adjust the detector's integration time to achieve a signal within the linear range of the instrument.

Troubleshooting

Problem	Possible Cause	Solution
No signal	Low transfection efficiency	Optimize transfection conditions using a visual transfection control (e.g., a plasmid over-expressing a fluorescent protein)
		Verify plasmid DNA quality using restriction digestion and agarose gel electrophoresis. High-quality plasmid DNA should be mostly supercoiled
		Use actively dividing, low-passage cells
		Use a different cell type
	No promoter induction	Incubate cells under promoter-specific inducing conditions
		Incubate the cells for a longer time after treatment
		Change growth conditions to improve expression
		Use a different promoter or cell type
	Firefly Luc One-Step Substrate auto-oxidized	Protect substrate from light and air. Store Firefly Luc One-Step Substrate Solution at -80°C
		Prepare Working Solution immediately before use and protect from light
	Low luciferase expression	Use a different promoter or growth conditions to improve expression
		Increase the integration time on the instrument
		Plate higher cell number
Signal is high	High luciferase expression	Reduce incubation time before collecting samples
		Decrease the integration time on the instrument
		Decrease the amount of plasmid transfected into cells or decrease cell number
		Avoid repeated freezing and thawing of the sample
	Control sample is contaminated	Change pipette tips after each well

Additional Information

- Visit www.thermofisher.com for additional information relating to this product including http://www.piercenet.com/method/luciferase-reporters.

Related Thermo Scientific Products

16155	**pMCS-Firefly Luc**
16156	**pCMV-Firefly Luc**
16157	**pTK-Firefly Luc**
85255	**Pierce Red Firefly LentiLuc Packaging Kit**
85272-3	**Pierce CMV-Red Firefly LentiLuc Particles**
85274-5	**Pierce CMV-Red Firefly LentiLuc Particles**
R0533	**TurboFect *in vitro* Transfection Reagent**
88951-2	**alamarBlue™ Cell Viability Assay Reagent**
88853-4	**Pierce LDH Cytotoxicity Assay Kit**
28374	**Modified Dulbecco's PBS Packs**

General Reference

Tatsumi, H., *et al.* (1989) Luciferase cDNA from Japanese firefly, *Luciola cruciata*: cloning, structure and expression in *Escherichia coli. J Biolumin Chemilumin* **3(2):**75-8.