INSTRUCTIONS

Pierce® Firefly Luciferase Glow Assay Kit

16176 16177 2368.0

Number	Description
16176	**Pierce Firefly Luciferase Glow Assay Kit,** sufficient reagents to perform 100 assays for firefly luciferase activity in cultured cell lysate **Kit Contents:** **Firefly Glow Assay Buffer,** 5mL, store at -20°C **D-Luciferin, Lyophilized,** 3mg, store at 4°C **2X Cell Lysis Buffer,** 6mL, store at room temperature
16177	**Pierce Firefly Luciferase Glow Assay Kit**, sufficient reagents to perform 1000 assays for firefly luciferase activity in cultured cell lysate **Kit Contents:** **Firefly Glow Assay Buffer,** 50mL, store at -20°C **D-Luciferin, Lyophilized,** 30mg, store at 4°C **2X Cell Lysis Buffer,** 60mL, store at room temperature

Storage: Upon receipt store kit at -20°C or store individual components as indicated above. Kit is shipped on dry ice.

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Introduction

The Thermo Scientific Pierce Firefly Luciferase Glow Assay provides a highly sensitive system for detecting intracellular luciferase activity from promoter or pathway activation in mammalian cell culture experiments. The bioluminescent signal produced by firefly luciferase results from the oxidation of D-Luciferin (Figure 1). The glow kit produces an extended light signal correlating with the amount of firefly protein expressed, which is proportional to the activity of the promoter for firefly expression.

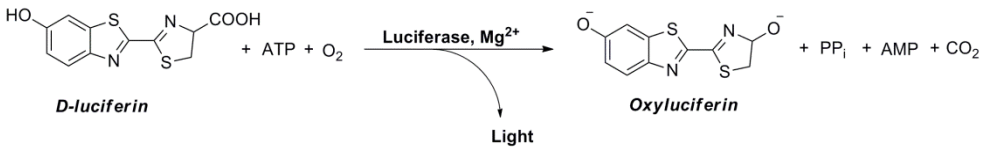


Figure 1. **Chemical reaction of luciferin and red firefly luciferase.** Light, with an emission maximum of 613nm, is produced from the oxidation of D-luciferin by red firefly luciferase in an ATP-dependent reaction.

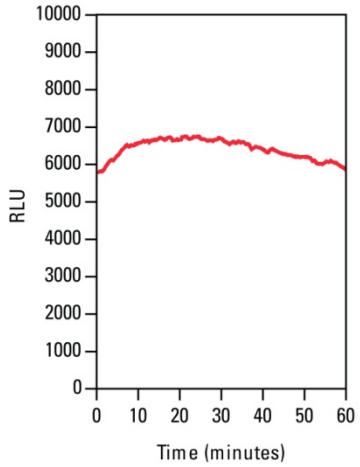
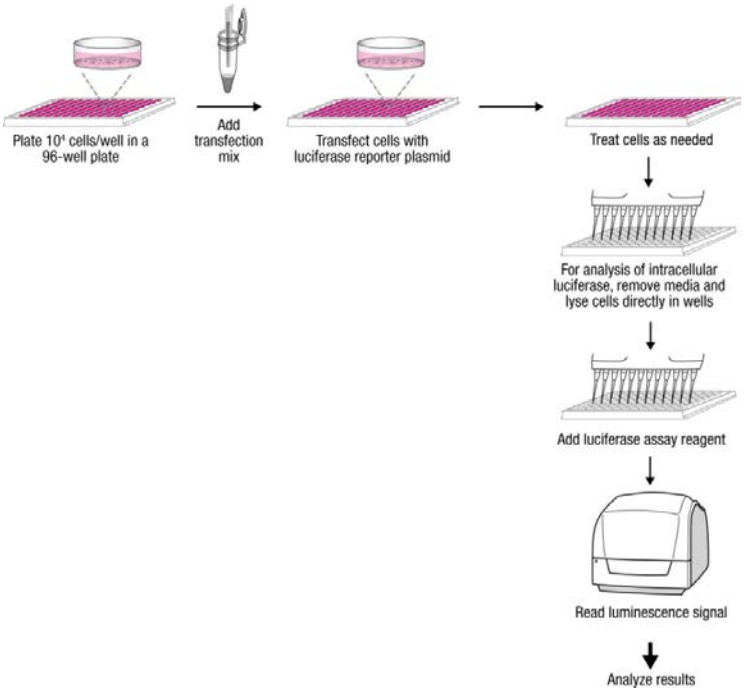


Figure 2. Thermo Scientific Pierce Firefly Luciferase Glow Assay signal stability over time. Light output using the Pierce Firefly Luciferase Glow Assay in HEK293 cell lysate decays slowly over time in a glow-type reaction.

Procedure Summary



Important Product Information

- For long-term use, store D-Luciferin (lyophilized) at 4°C protected from light.
- Store Firefly Luciferase Glow Assay Working Solution (Working Solution) protected from light. Working Solution must be at room temperature (20-25°C) before use and is stable for up to four hours at room temperature.
- To avoid cross-contamination, use a new disposable pipette tip for each transfer. Always use a new disposable reagent reservoir for each reagent.
- Firefly luciferase in cell lysate is subject to degradation. Store firefly luciferase-containing cell lysate on ice; perform assays immediately following cell lysis. Addition of protease inhibitors to the cell lysis buffer can increase firefly luciferase stability (see the Related Thermo Scientific Products Section).
- 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 with those reserved for additional plates.
- Individual components might contain corrosives and/or preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please 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 for propagating mammalian cells in culture
- Reagents and materials for transfection of plasmid DNA into mammalian cells (e.g., Thermo Scientific TurboFect Transfection Reagent, Product No. R0532-4)
- Modified Dulbecco's Phosphate-buffered saline (DPBS) (e.g., Thermo Scientific BupH Modified Dulbecco's PBS, 8mM sodium phosphate, 2mM potassium phosphate, 140mM sodium chloride, 10mM potassium chloride; pH 7.4; Product No. 28374)
- Laboratory platform shaker
- Pipettes and/or liquid handling equipment
- Luminometer or other luminescence-monitoring instrument
- White or black opaque, 96- or 384-well microplates

Material Preparation

100X D-Luciferin Stock Solution	For 100 reactions, reconstitute lyophilized D-Luciferin pellet in 50µL of Firefly Glow Assay Buffer. Store at -20°C for up to two months.
Working Solution	For 100 reactions, add 50µL of 100X reconstituted D-Luciferin to 5mL of Firefly Glow Assay Buffer. Use 50µL of the Working Solution per reaction. **Note:** Dilute Thermo Scientific Pierce Firefly Signal Enhancer (100X) (Product No. 16180) to 1X in Working Solution.
1X Cell Lysis Buffer	Dilute 2X Cell Lysis Buffer with an equal volume of ultrapure water.

Procedure for Firefly Luciferase Glow Assay

A. Cell Transfection

1. Plate ~10,000 cells/well in a 96-well plate. Incubate plates overnight at 37°C in 5% CO_2. If using a different plate size, adjust the cell number accordingly. Use only cells growing in log phase at a passage number ≤ 15.

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

2. Use a standard protocol to transfect mammalian cells with a firefly luciferase plasmid.
3. Incubate cells for 16-72 hours at 37°C in 5% CO_2 in a cell culture incubator.
4. Proceed with the individual experimental protocol for cell treatment.

B. Cell Lysis

1. Aspirate media from the cells, rinse once with 100µL/well of 1X DPBS buffer (Thermo Scientific BupH Modified Dulbecco's PBS, Product No. 28374), aspirate DPBS and add 50-100µL/well of 1X Cell Lysis Buffer. Do not disturb the cell monolayer during the DPBS rinse.
2. Shake the plate on a platform shaker at moderate speed for 15 minutes. Check for complete cell lysis using a light microscope. If lysis is incomplete, continue shaking the plate for 15 additional minutes.

C. Firefly Luciferase Glow Assay

1. Program the luminometer.
2. Add 10-20µL/well of cell lysate to a white or black, opaque 96-well plate.
3. Add 50µL of Working Solution to each well.
4. Wait 10 minutes for signal stabilization and detect the light output.

Troubleshooting

Problem	Possible Cause	Solution
No signal or low 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; use only transfection grade DNA
		Use actively dividing, low passage cells
		Use a different cell type
	No promoter activity	Use conditions known for promoter activation
		Incubate cells for a longer time
		Change growth conditions to improve expression
		Use a different promoter
	D-Luciferin auto-oxidized	Protect substrate from light and air and maintain 100X D-Luciferin at -20°C
		Prepare new Working Solution if used longer than 4 hours
	Low luciferase expression	Use Pierce Firefly Signal Enhancer (100X) (Product No. 16180)
		Lyse cells in a smaller volume of 1X Cell Lysis Buffer
		Use a different promoter or growth conditions to improve expression
		Increase the integration time on the instrument
		Scale-up the volume of sample and reagent per well
	Degraded luciferase protein	Store cell lysates on ice and perform assays immediately following cell lysis

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High signal	High luciferase expression	Reduce incubation time before collecting samples
		Decrease the integration time on the instrument
		Dilute the sample **Note:** A low sample volume can increase assay variability; dilute the sample and use the recommended volume of 10-20µL per assay
High background signal	Control sample was contaminated	Incubate cells for a longer time

Related Thermo Scientific Products

See our website for a complete list of related luciferase products.

16180	**Pierce Firefly Signal Enhancer**
16155	**pMCS-Red Firefly Luc**
16156	**pCMV-Red Firefly Luc**
16157	**pTK-Red Firefly Luc**
16189	**Pierce Luciferase Cell Lysis Buffer**
R0532-4	**TurboFect Transfection Reagent**
28374	**BupH™ Modified Dulbecco's PBS Packs, 40 packs**
28344	**20X Modified Dulbecco's PBS Buffer**
78425	**Halt Protease Inhibitor Single-use Cocktail, EDTA-free**

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.