

# Pierce<sup>®</sup> Renilla Luciferase Flash Assay Kit

16164 16165

2363.0

Number	Description
16164	<p><b>Pierce Renilla Luciferase Flash Assay Kit</b>, sufficient reagents to perform 100 assays for <i>Renilla</i> luciferase activity in cultured cell lysate</p> <p><b>Kit Contents:</b></p> <p><b>Renilla Flash Assay Buffer</b>, 5mL, store at 4°C</p> <p><b>Coelenterazine (100X)</b>, 50µL, store at -80°C</p> <p><b>2X Cell Lysis Buffer</b>, 6mL, store at room temperature</p>
16165	<p><b>Pierce Renilla Luciferase Flash Assay Kit</b>, sufficient reagents to perform 1000 assays for <i>Renilla</i> luciferase activity in cultured cell lysate</p> <p><b>Kit Contents:</b></p> <p><b>Renilla Flash Assay Buffer</b>, 50mL, store at 4°C</p> <p><b>Coelenterazine (100X)</b>, 0.5mL, store at -80°C</p> <p><b>2X Cell Lysis Buffer</b>, 60mL, store at room temperature</p>

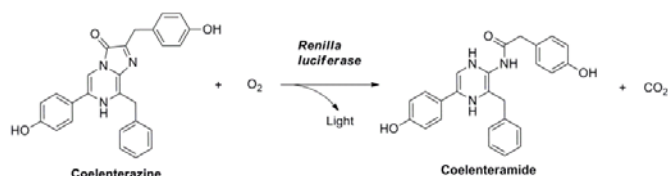
**Storage:** Upon receipt store kit at -80°C or store individual components as indicated above. Kit is shipped on dry ice.

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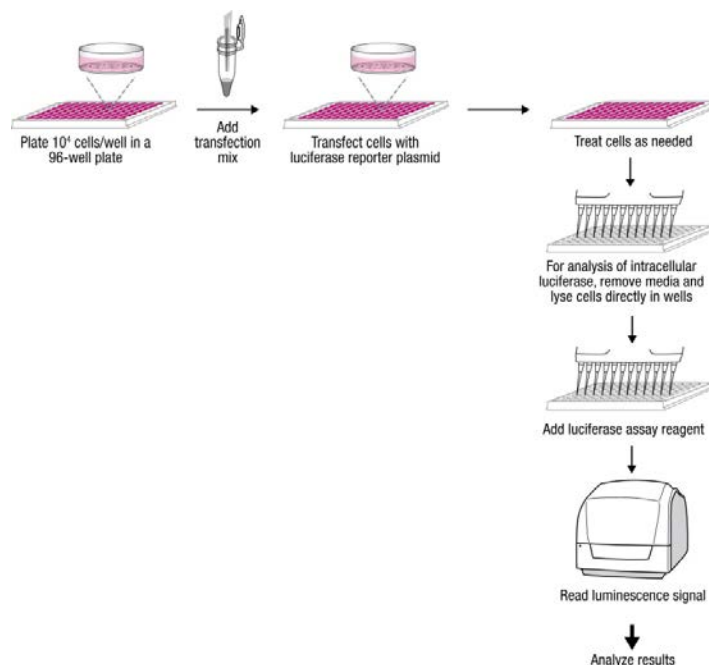
## Introduction

The Thermo Scientific Pierce Renilla Luciferase Flash 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 green *Renilla* luciferase results from the oxidation of coelenterazine (Figure 1). This reaction does not require adenosine triphosphate (ATP) or other cofactors. The light output correlates with the amount of green *Renilla* protein expressed, which is proportional to the activity of the promoter for green *Renilla* expression.



**Figure 1. Chemical reaction of coelenterazine and green *Renilla luciferase*.** Light, with an emission maximum of 535nm, is produced from the oxidation of coelenterazine and green *Renilla luciferase*.

## Procedure Summary



## Important Product Information

- For long-term use, store Coelenterazine (100X) at -80°C protected from light. Briefly centrifuge tubes of Coelenterazine (100X) before use.
- Store *Renilla* Luciferase Flash 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 8 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.
- Green *Renilla* luciferase protein is significantly more stable than firefly luciferase protein in cell culture lysate. Samples may be kept at room temperature for same-day testing or frozen at -80°C for long-term use. Addition of protease inhibitors to the lysate has been shown to increase the stability of green *Renilla* luciferase protein (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.

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## 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  
**Note:** Use a luminometer equipped with reagent injectors to perform > 24 assays. (Optional)
- White or black opaque, 96- or 384-well microplates

## Material Preparation

Working Solution	For 100 reactions, add 50 $\mu$ L of 100X Coelenterazine to 5mL of Renilla Flash Assay Buffer. Use 50 $\mu$ L of the Working Solution per reaction. <b>Note:</b> If using a luminometer with injectors, prepare sufficient reagent to prime the pumps in addition to reagent required for the assay.
1X Cell Lysis Buffer	Dilute 2X Cell Lysis Buffer with an equal volume of ultrapure water.

## Procedure for Renilla Luciferase Flash Assay

### A. Cell Transfection

1. Plate ~10,000 cells/well in a 96-well plate. Incubate plates overnight at 37°C in 5% CO<sub>2</sub>. If using a different plate size, adjust the cell number accordingly. Use only cells growing in log phase at a passage number  $\leq$  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 *Renilla* luciferase plasmid.
3. Incubate cells for 16-72 hours at 37°C in 5% CO<sub>2</sub> in a cell culture incubator.
4. Proceed with the individual experimental protocol for cell treatment.

### B. Cell Lysis

1. Rinse once with 100 $\mu$ L/well of 1X DPBS buffer (Thermo Scientific BupH Modified Dulbecco's PBS, Product No. 28374), aspirate DPBS and add 50-100 $\mu$ L/well of 1X Cell Lysis Buffer. Do not disturb the cell monolayer during the transfer and wash steps.
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. Renilla Luciferase Flash Assay

1. Add 10-20 $\mu$ L/well of cell lysate to a white or black, opaque 96-well plate.
2. Program the luminometer; if using an injector, prime the injector with Working Solution.
3. Add 50 $\mu$ L of Working Solution to each well.
4. Immediately after adding the reagent, detect the light output.

**Note:** Follow the manufacturer's recommendations for using injector velocity to obtain a uniform coating of liquid in the well. Adjust the detector's integration time to achieve a signal within the linear range of the instrument.

## 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
	Coelenterazine auto-oxidized	Protect substrate from light and air and maintain 100X Coelenterazine at -80°C
		Prepare new Working Solution if used longer than 8 hours
	Low luciferase expression	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  Note: Addition of protease inhibitors to the cell lysis buffer can increase the stability of the <i>Renilla</i> luciferase protein (see Related Thermo Scientific Products)	
High signal	High luciferase expression	Reduce incubation time before collecting samples
		Decrease the integration time on the instrument
		Dilute the sample <b>Note:</b> A low sample volume can increase assay variability; dilute the sample and use the recommended volume of 10-20µL per assay
High background signal	Nonspecific oxidation of coelenterazine	Use less serum in the cell culture media
		Avoid repeated freezing and thawing of the sample
	Control sample was contaminated	Change pipette tips after each well

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## Related Thermo Scientific Products

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

16152	pMCS-Green Renilla Luc
16153	pCMV-Green Renilla Luc
16154	pTK-Green Renilla 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

Lorenz, W.W., *et al.* (1991). Isolation and expression of cDNA encoding *Renilla reniformis* luciferase. *Proc Natl Acad Sci USA* **88**:4438-42.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

**No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).**

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at [www.thermoscientific.com/pierce](http://www.thermoscientific.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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