

# Pierce™ Gaussia Luciferase Flash Assay Kit

16158 16159

2361.1

Number	Description
16158	<p><b>Pierce Gaussia Luciferase Flash Assay Kit</b>, sufficient reagents to perform 100 assays for <i>Gaussia</i> luciferase activity in media and cultured cell lysate</p> <p><b>Kit Contents:</b></p> <p><b>Gaussia 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>
16159	<p><b>Pierce Gaussia Luciferase Flash Assay Kit</b>, sufficient reagents to perform 1000 assays for <i>Gaussia</i> luciferase activity in media and cultured cell lysate</p> <p><b>Kit Contents:</b></p> <p><b>Gaussia 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>

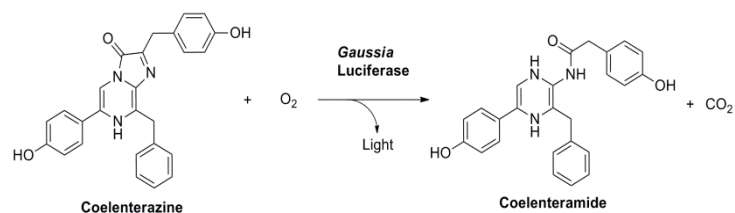
**Storage:** Upon receipt store kit at -80°C or store individual components as indicated above. Kit is shipped on dry ice.

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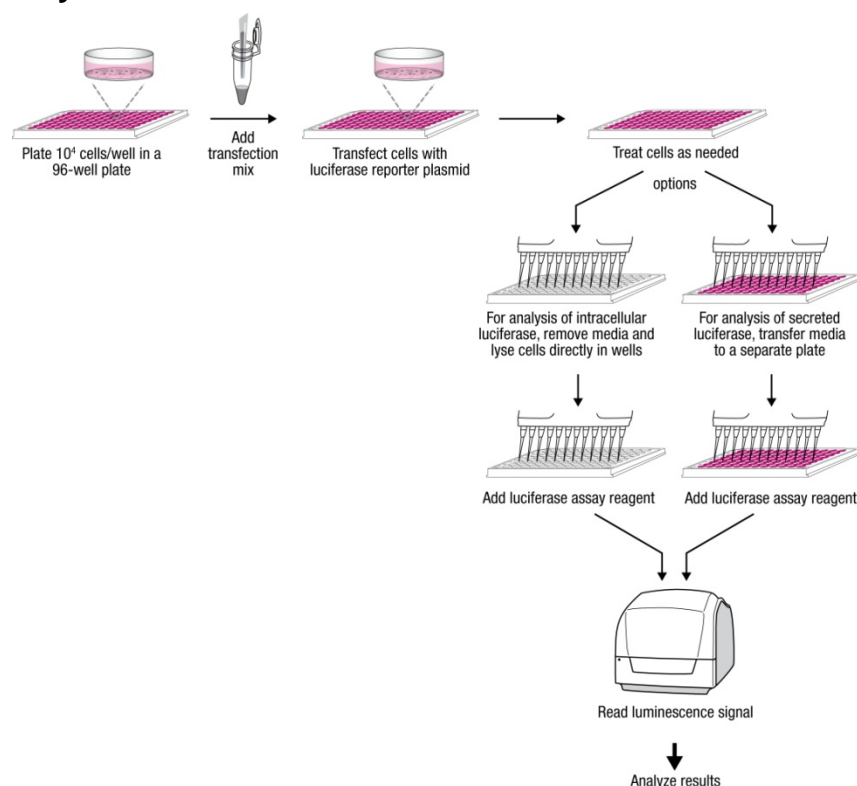
## Introduction

The Thermo Scientific Pierce Gaussia Luciferase Flash Assay provides a highly sensitive system for detecting intracellular and secreted luciferase activity from promoter or pathway activation in mammalian cell culture experiments. *Gaussia* luciferase has greater protein stability and signal brightness than firefly and native *Renilla* luciferase. The bioluminescent signal produced by *Gaussia* 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 *Gaussia* protein expressed, which is proportional to the activity of the promoter for *Gaussia* expression.



**Figure 1. Chemical reaction of coelenterazine and *Gaussia* luciferase.** Light, with an emission maximum of 485nm, is produced from the oxidation of coelenterazine by *Gaussia* 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. Coelenterazine (100X) is volatile; seal tube tightly after use.
- Store Gaussia 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 four hours at room temperature.
- Gaussia* luciferase protein is significantly more stable than red firefly luciferase protein. Keep samples at room temperature for same-day testing or -80°C for long-term use.
- To avoid cross-contamination, use a new disposable pipette tip for each transfer. Always use a new disposable reagent reservoir for each reagent.
- 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. R0533)
- 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 Gaussia Flash Assay Buffer. Use 50 $\mu$ L of the Working Solution per reaction.

**Note:** If using a luminometer with injectors, prepare sufficient reagent to prime the pumps in addition to reagent required for assay.

**1X Cell Lysis Buffer** Dilute 2X Cell Lysis Buffer with an equal volume of ultrapure water.

## Procedure for Gaussia 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 *Gaussia* 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.

**Note:** Replace the cell culture media before cell treatment to remove any secreted *Gaussia* luciferase.

### B. Collection of Media and/or Cell Lysis

1. Remove 10-20 $\mu$ L of media from the transfected cells within 72 hours after transfection.

**Note:** Collect media at different times to monitor changes in luciferase expression; media can be collected at multiple time points without sacrificing the cells.

2. Lyse cells to monitor intracellular luciferase activity. Rinse the cells with 100 $\mu$ L/well of 1X DPBS buffer, 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.

**Note:** The lysis buffer volume is for a 96-well plate. If using a different plate size, adjust the volume accordingly.

3. Rotate 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 an additional 15 minutes.

### C. Gaussia Luciferase Flash Assay

1. Add 10-20 $\mu$ L/well of cell lysate or media 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	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 or low 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 signal in media	Insufficient luciferase accumulation in media	Incubate cells for a longer time
	Low luciferase expression	Use less media per well during the experiment
		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
	Treatment interfered with cellular secretory pathway	Transfect cells with a plasmid for constitutive expression of luciferase (i.e., pTK-Gaussia Luc or pCMV-Gaussia Luc); determine if luciferase actively expresses in media without treatment. Add treatment; determine if there is a corresponding drop in luciferase activity from the constitutively-expressed plasmid

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Low signal in lysate	Non-optimized lysis buffer used	Assay luciferase activity in the media to confirm good expression of luciferase
		Use only the provided lysis buffer
	Low luciferase expression	Lyse cells in 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 volume of sample and reagent per well	
High saturating signal	High luciferase expression	Reduce incubation time before collecting samples
		Decrease the integration time on the instrument
		Dilute the sample: for secreted <i>Gaussia</i> , dilute the sample using media from the cell culture; for cell lysate, dilute the sample using lysis buffer <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 is contaminated	Use new sample
		Change pipette tips after each well

## Related Thermo Scientific Products

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

<b>16146</b>	<b>pMCS-Gaussia Luc</b>
<b>16147</b>	<b>pCMV-Gaussia Luc</b>
<b>16148</b>	<b>pTK-Gaussia Luc</b>
<b>16190</b>	<b>pMCS-Gaussia-Dura Luc</b>
<b>16191</b>	<b>pCMV-Gaussia-Dura Luc</b>
<b>16192</b>	<b>pTK-Gaussia-Dura Luc</b>
<b>16189</b>	<b>Pierce Luciferase Cell Lysis Buffer</b>
<b>R0533</b>	<b>TurboFect Transfection Reagent</b>
<b>28374</b>	<b>Modified Dulbecco's PBS BupH Packs</b>
<b>28344</b>	<b>20X Modified Dulbecco's PBS Buffer</b>
<b>87785</b>	<b>Halt Protease Inhibitor Cocktail, EDTA-free</b>

## General References

Szent-Gyorgyi, C., *et al.* (1999). Cloning and characterization of new bioluminescent proteins. Part of the SPIE Conference on Molecular Imaging: Reporters, Dyes, Markers, and Instrumentation. San Jose, CA. *Proc SPIE* **3600**:4-11.

Tannous, B.A., *et al.* (2005). Codon-optimized *Gaussia* luciferase cDNA for mammalian gene expression in culture and *in vivo*. *Molec Ther* **11**:435-43.

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Limited Use Label License: Gaussia Luciferase (Thermo Scientific Product Nos. 16146, 16147, 16148, 16190, 16191, 16192)

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In addition, all Recipients must either

a). use Thermo Scientific bioluminescent assay reagents purchased from Thermo Fisher Scientific or its distribution channels (listed below), for all determinations of Gaussia bioluminescence activity.

Product Nos. 16158, 16159: Pierce® Gaussia Luciferase Flash Assay Kit

Product Nos. 16160, 16161: Pierce® Gaussia Luciferase Glow Assay Kit

Product Nos. 16181, 16182: Pierce® Gaussia-Firefly Luciferase Dual Assay Kit

Product Nos. 16187, 16188: Pierce® Cypridina-Gaussia Luciferase Dual Assay Kit

or

b). contact [proteomics.licensing@thermofisher.com](mailto:proteomics.licensing@thermofisher.com), Pierce Biotechnology, Inc., 3747 North Meridian Road, Rockford, IL 61101, in order to obtain a license.

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