INSTRUCTIONS

# Pierce Gaussia Luciferase Glow Assay Kit

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16160 16161

| Number | Description |
|---|---|
| **16160** | **Pierce Gaussia Luciferase Glow Assay Kit,** sufficient reagents to perform 100 assays for *Gaussia* luciferase activity in media and cultured cell lysate<br>**Kit Contents:**<br>**Gaussia Glow Assay Buffer,** 5mL, store at -20°C<br>**Coelenterazine (100X),** 50µL, store at -80°C<br>**2X Cell Lysis Buffer,** 6mL, store at room temperature |
| **16161** | **Pierce Gaussia Luciferase Glow Assay Kit**, sufficient reagents to perform 1000 assays for *Gaussia* luciferase activity in media and cultured cell lysate<br>**Kit Contents:**<br>**Gaussia Glow Assay Buffer,** 50mL, store at -20°C<br>**Coelenterazine (100X),** 0.5mL, store at -80°C<br>**2X Cell Lysis Buffer,** 60mL, store at room temperature |

**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 Glow 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.

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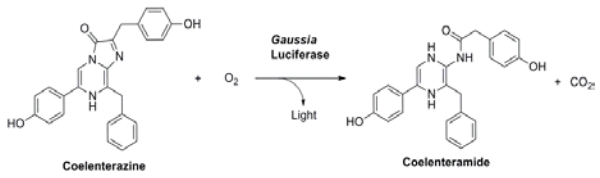



**Figure 1**. **Chemical reaction of coelenterazine and *Gaussia* luciferase.** Light, with an emission maximum of 485nm, is produced from the oxidation of coelenterazine by *Gaussia* luciferase.

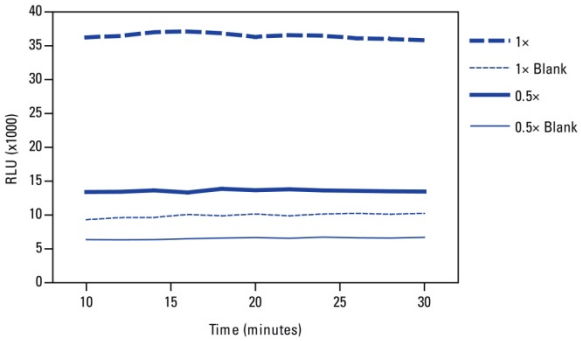
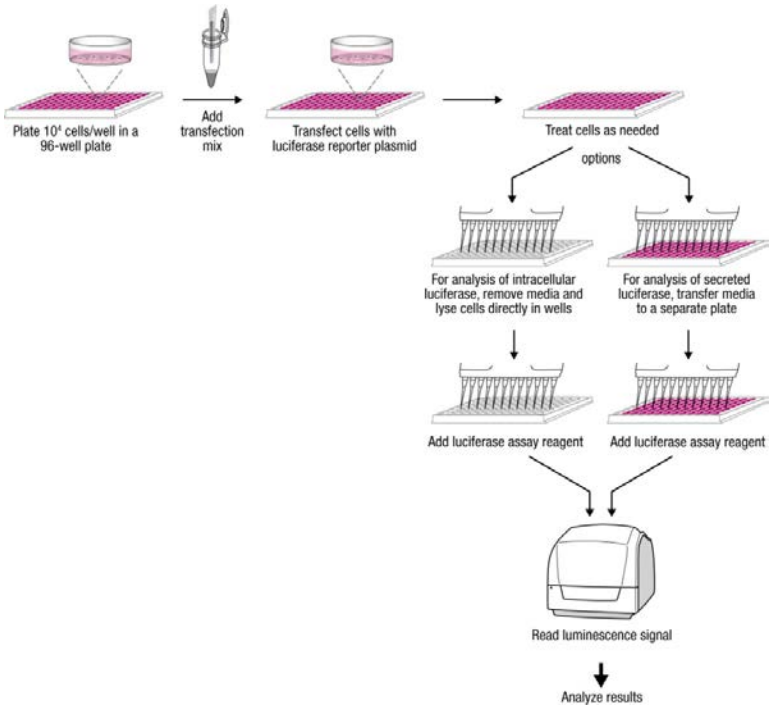



**Figure 2. Thermo Scientific Pierce Gaussia Luciferase Glow Assay signal stability over time.** Media from HEK293 cells transfected with pTK-Gaussia-Dura plasmid was assayed for *Gaussia* luciferase activity using the Pierce Gaussia Luciferase Glow Assay Kit. The graph shows glow-type signal for two different substrate concentrations with each detected 10 minutes after addition of the assay reagent. Increasing the substrate concentration increases the signal; however, high amounts of luciferase (e.g., as expressed from strong promoters like CMV) may decrease signal stability after 25 minutes of reaction (data not shown). Nonspecific background at various substrate concentrations is represented by the dotted lines.

## 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 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.
- *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
- White or black opaque, 96- or 384-well microplates

## Material Preparation

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| Working Solution | For 100 reactions, add 50µL of 100X Coelenterazine to 5mL of Gaussia Glow Assay Buffer. Use 50µL of the Working Solution per reaction. |
| 1X Cell Lysis Buffer | Dilute 2X Cell Lysis Buffer with an equal volume of ultrapure water. |

## Procedure for Gaussia 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 *Gaussia* 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.

   **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 10-20µL 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 100µL/well of 1X DPBS buffer, aspirate DPBS and add 50-100µL/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 Glow Assay**

1. Program the luminometer.
2. Add 10-20µL/well of cell lysate or media 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 | 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 *Gaussia*, dilute the sample using media from the cell culture; for cell lysate, dilute the sample using lysis buffer<br>**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 | 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.**

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| **16190** | **pMCS-Gaussia-Dura Luc** |
| **16191** | **pCMV-Gaussia-Dura Luc** |
| **16192** | **pTK-Gaussia-Dura Luc** |
| **16146** | **pMCS-Gaussia Luc** |
| **16147** | **pCMV-Gaussia Luc** |
| **16148** | **pTK-Gaussia Luc** |
| **16189** | **Pierce™ Luciferase Cell Lysis Buffer** |
| **R0532-4** | **TurboFect Transfection Reagent** |
| **28374** | **Modified Dulbecco's PBS BupH Packs** |
| **28344** | **20X Modified Dulbecco's PBS Buffer** |

### 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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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, Pierce Biotechnology, Inc., 3747 North Meridian Road, Rockford, IL 61101, in order to obtain a license.

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