INSTRUCTIONS

Pierce® Cypridina Luciferase Flash Assay Kit

16168 16169 2365.0

Number	Description
16168	**Pierce Cypridina Luciferase Flash Assay Kit**, sufficient reagents to perform 100 assays for *Cypridina* luciferase activity in media and cultured cell lysate **Kit Contents:** **Cypridina Flash Assay Buffer**, 5mL, store at 4°C **Vargulin (100X)**, 50µL, store at -20°C **2X Cell Lysis Buffer**, 6mL, store at room temperature
16169	**Pierce Cypridina Luciferase Flash Assay Kit**, sufficient reagents to perform 1000 assays for *Cypridina* luciferase activity in media and cultured cell lysate **Kit Contents:** **Cypridina Flash Assay Buffer,** 50mL, store at 4°C **Vargulin (100X)**, 0.5mL, store at -20°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 Cypridina Luciferase Flash Assay provides a highly sensitive system for detecting secreted or intracellular luciferase activity from promoter or pathway activation in mammalian cell culture experiments. *Cypridina* luciferase has greater protein stability and signal brightness than firefly and native *Renilla* luciferase. The bioluminescent signal produced by *Cypridina* luciferase results from the oxidation of vargulin (Figure 1). This reaction does not require adenosine triphosphate (ATP) or other cofactors. The light output correlates with the amount of *Cypridina* protein expressed, which is proportional to the activity of the promoter for *Cypridina* expression.

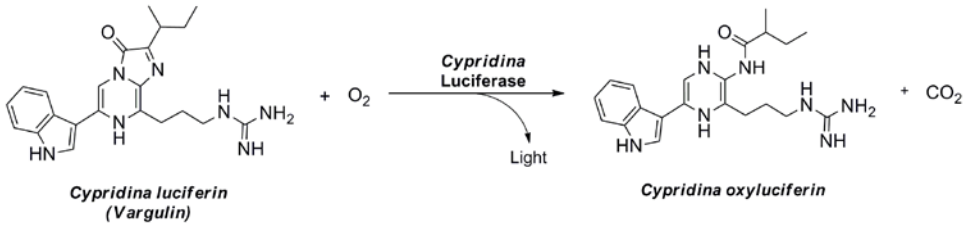
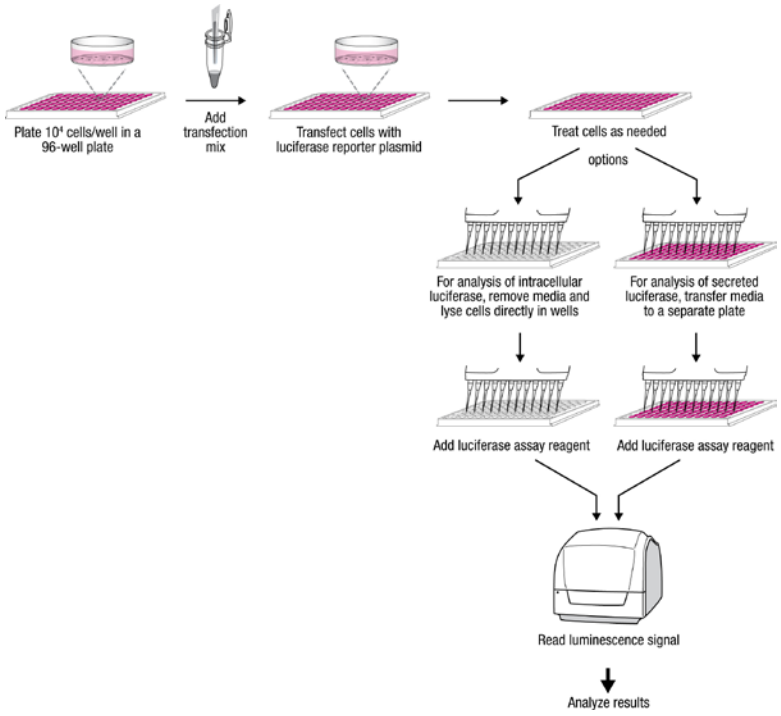


Figure 1. Chemical reaction of Vargulin and *Cypridina* luciferase. Light, with an emission maximum of 463nm, is produced from the oxidation of vargulin by *Cypridina* luciferase.

Procedure Summary



Important Product Information

- For long-term use, store Vargulin (100X) at -20°C protected from light. Briefly centrifuge tubes of Vargulin (100X) before use. Vargulin (100X) is volatile; seal tube tightly after use.
- Store Cypridina 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 two hours at room temperature.
- Cypridina luciferase protein is significantly more stable than 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 assay plates

Material Preparation

Working Solution	For 100 reactions, add 50µL of 100X Vargulin to 5mL of Cypridina Flash Assay Buffer. Use 50µL 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 the assay.
1X Cell Lysis Buffer	Dilute 2X Cell Lysis Buffer with an equal volume of ultrapure water.

Procedure for Cypridina 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_2. If using a different plate size, adjust the cell number accordingly. Use only cells growing in log phase at a passage number of ≤ 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 *Cypridina* 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 *Cypridina* 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. Cypridina Luciferase Flash Assay

1. Add 10-20µL/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 50µL 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	Incubate cells under inducing conditions (promoter specific)
		Incubate cells for a longer time
		Change growth conditions to improve expression
		Use a different promoter
	Vargulin auto-oxidized	Protect substrate from light and air and maintain 100X Vargulin at -20°C
		Prepare new Working Solution if used longer than 2 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-Cypridina Luc or pCMV-Cypridina 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	Majority of luciferase is secreted	Assay luciferase activity in the media
	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 volume of sample and reagent per well
High signal	High expression of luciferase	Reduce incubation time before collecting samples
		Decrease the integration time on the instrument
		Dilute the sample: for secreted *Cypridina*, dilute the sample using media from the cell culture; for cell lysate, dilute the sample using lysis buffer **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 vargulin	Use less serum in the cell culture media **Note:** Albumin can increase the auto-oxidation of vargulin
		Avoid repeated freezing and thawing of the sample
	Control sample is contaminated	Change pipette tips after each well

Related Thermo Scientific Products

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

16149	**pMCS-Cypridina Luc**
16150	**pCMV-Cypridina Luc**
16151	**pTK-Cypridina Luc**
16189	**Pierce Luciferase Cell Lysis Buffer**
R0533	**TurboFect Transfection Reagent**
28374	**BupH Modified Dulbecco's PBS Packs,** 40 packs
28344	**20X Modified Dulbecco's PBS**

General Reference

Nakajima, Y., *et al.* (2004). cDNA cloning and characterization of a secreted luciferase from the luminous Japanese ostracod, *Cypridina noctiluca*. *Biosci Biotechnol Biochem* **68(3):**565-70.