ab189814

Gaussia Luciferase Cellular Assay Kit

Instructions for Use

For the detection of Gaussia Luciferase using Cellular or Solution Assay

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

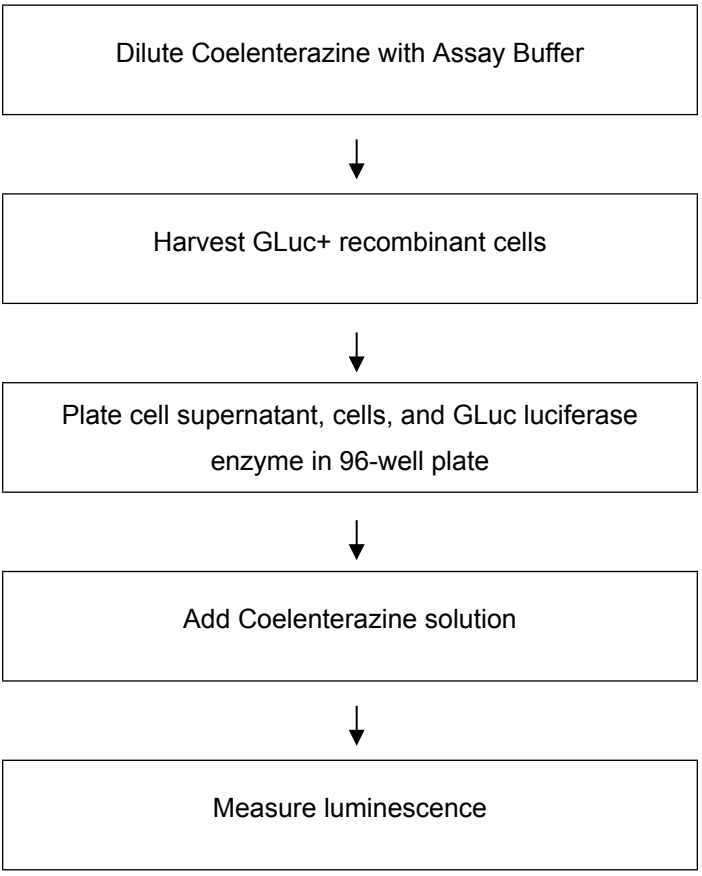
Gaussia Luciferase is a new reporter gene isolated from the marine copepod *Gaussia princeps*. Gaussia luciferase can be expressed in mammalian cells using commercially available reporter plasmids. This luciferase, which does not require ATP, catalyzes the oxidation of the substrate coelenterazine in a reaction that emits light (at 470 nm), and has considerable advantages over other reporter genes. Gaussia Luciferase possesses a natural secretory signal and upon expression is secreted into the cell medium of cells grown in culture. Therefore, cell lysis is not necessary for assaying expression levels. In addition, the gene product has a molecular weight of only 19.9 kDa (185 AA) making it one of the smallest known luciferases. The enzyme also has a broad pH optimum centered at 7.7, with activity dependent upon the concentration of monovalent cations. The enzyme is resistant to decomposition due to exposure to heat, cold, strongly acidic or basic conditions. Analysis of the gene sequence indicates a secretory signal that functions in both prokaryotes and eukaryotes. Most importantly, Gaussia luciferase is reported to generate over 1000-fold higher bioluminescent signal intensity, compared to Firefly and *Renilla* Luciferases, making it an ideal transcriptional reporter for cell culture assays. The secreted protein is very stable and can be stored for several days at 4°C without significant loss of activity. The substrate, coelenterazine, is common to a number of marine bioluminescent reactions, including those

from *Renilla*, *Aequorea* and *Watesenia*. In some of these reactions it is utilized as a simple substrate being catalytically turned over in the bioluminescent reaction, while in others, such as in the photoprotein systems of Mneiopsis, it is incorporated as part of the photoprotein.

Abcam's Gaussia Luciferase Cellular Assay Kit (ab189814) contains enough reagents for 100 tests using a microtiter plate format and includes buffers, solvents and a detailed protocol for use in quickly measuring the luciferase activity in mammalian cell culture.

Emission: 470 nm.

2. Protocol Summary



3. Kit Contents

Components	Amount	Storage
Coelenterazine *	1 vial	Store at or below -20°C Light sensitive Avoid breathing dust/fumes
Assay Buffer	5 mL	Store cold at 4°C
Dilution Buffer	5 mL	Store cold at 4°C
Lysis Buffer	5 mL	Store cold at 4°C

4. Storage and Handling

Fluorescent reagents, standards and buffers should be handled with care, kept cold (ice-bath) when not in use, and stored at 4°C. In case of contact with skin or eyes wash thoroughly with soap and cold water. High background fluorescence readings for blank samples will indicate decomposition. Upon arrival, all coelenterazine solutions should be stored at -80°C for long periods of time or at -20°C if used within a few days. The working solution should be kept on ice during preparation of the substrate solutions.

5. Additional Materials Required

- 96 or 384-well black wall/clear bottomed microplates
- PBS or HHBS
- CO_2 Incubator
- Ice
- Triton X-100
- Methanol
- Luminometer or microplate reader that can measure luminescence

6. Assay Protocol

A. Details for Performing the Assay

The Gaussia luciferase GLuc assays can be grouped into two general classes based on whether the luciferase activity is measured in cell culture using media from intact cells or tissues (Cellular Assay) or from purified enzyme systems (Solution Assay). In both these types of assay conditions, it is recommended that standard or control assays are performed simultaneously either using purified enzyme if possible, as well as no-enzyme blank samples. The light output from the luciferase assay can be measured as peak luminescence or as an integrated light output, which includes peak luminescence (typically 1-60 sec. duration). Both of these values should be proportional to the initial luciferase concentration. The light emission from the enzyme reaction peaks within seconds at a maximum of 470 nm (blue-green color) and using the purified enzyme in the presence of excess substrate(s), the light output is proportional to the concentration of luciferase present.

B. Protocol

The sensitivity of Gaussia luciferase assay can vary depending on several factors described in assay conditions below. The optimal luciferase assay conditions will need to be measured in each experiment to maximize the luminescence signals.

- Warm the Coelenterazine solution (in deoxygenated, acidified methanol purged with N_2) to 0°C by placing in an ice-bath (approximately 20 minutes). It may be necessary to briefly centrifuge to collect solution at bottom of tube.

- Prepare a 1X Assay SSolution by adding the Coelenterazine solution to 5 mL Assay Buffer immediately before performing the assay. Rinse the Coelenterazine solution vial to make sure the complete transfer of its content. Keep this solution cold (ice-bath) throughout the analysis.

- For assay of GLuc+ recombinant cells, healthy cells are grown in culture (to approx. 80% confluent) under standard tissue culture conditions (CO_2 atmosphere, 37°C), under exponential growth conditions.

- Cell supernatant is removed and transferred to 96-well opaque microtiter plate wells (in triplicate) (25 µL per well).

- Combine 50 µL of 1X Assay Solution with cell supernatant from the GLuc luciferase recombinant cells (25 µL) in each well.

- Alternately, add the 1X Assay Solution (50 µL) to wells containing recombinant cells washed with PBS.

- Alternately, add the 1X Assay Solution (50 µL) to wells containing purified GLuc luciferase enzyme solution (25 µL) in separate wells as a positive control (if available). Purified GLuc should be prepared in Dilution Buffer.

- Measure luminescence using one of several different methods, such as luminometer, microtiter plate reader or CCD camera. Please refer to the manufacturer's recommended settings for these analyses. The luciferase activity reaches peak intensity quickly, and the immediate measurement of the luminescence signals is recommended. Integrate the light output for 20-60 seconds for final readings.

C. Solution Assay

1. Lysis conditions

The method of cell lysis may affect subsequent luciferase activity. Freeze-thaw cell lysis techniques may cause denaturation of the temperature sensitive luciferase enzyme and reduce its activity. A detergent mediated cell lysis technique employing the non-ionic detergent Triton X-100 is found to improve luciferase activity over freeze-thaw techniques by as much as 25-fold. This is due to both better stabilization of the enzyme and a more thorough extraction of the enzyme. A detergent mediated cell lysis buffer is provided in this kit. Modified detergent homogenization or sonication buffer solutions without the chelator EGTA have also been used. For mammalian tissue samples, homogenize approximately 5 mg of tissue (wet weight) to isolate 1 x 10^5 cells in 1.0 mL of Lysis Buffer, for assay. Enzyme recovery will be dependent upon tissue type and preparation conditions.

Extracts are centrifuged (13000 x *g*, 5-10 min.) and the supernatant solutions are removed and stored at –80°C. Vortex mixing of supernatant solutions prior to assay is suggested.

2. Assay conditions

Cell lysates or tissue homogenates are typically assayed as described below. A crude tissue homogenate or cell lysate (50 – 100 µL) is diluted with Dilution Buffer (usually 1 to 5 the sample volume) at 20°C and the light emission measured by one of the techniques described in Protocol section. For standardization, control reactions with purified enzyme are recommended, since the kinetics of the light reaction using a crude enzyme preparation is quite different from those observed with the purified crystalline luciferase enzyme. The difference in activity for the crude enzyme is often due to the presence of a number of contaminating enzymes, the presence of inorganic impurities or inhibitors.

For the standard assay, a linear response for light output with respect to luciferase protein concentration should be obtained, with intercept at the origin. Luciferase activity is expressed in light units (LU) per µg of protein. As described above, the light output can be measured as an integral of light emission over a defined time length (most common) or as the optimum light output (peak). In either case, standard curves should show a linear relationship.

Of course, additional analyses for luciferase expression (for example, gel electrophoresis) can be performed in which a standard Gaussia luciferase band (20 kDa) or band from your specific fusion protein with GLuc is also run.

7. Troubleshooting

1. What type of microtiter plate works best with this assay?

We do recommend using a black plate. This will inhibit fiber-optic type light transfer through the plate from sample to control wells. We also suggest skipping a row between samples to inhibit this cross-talk between wells. And many manufacturers of plates recommend not using the edge rows, although we haven't seen any problems with that here.

2. What is the optimum reading time?

The reading time will depend on your particular plate reader, but you should try to set the integration time to the maximum allowed by your instrument. We set our integration at the maximum of our plate reader, 100 seconds, but many plate readers (and many articles) have an integration time of much longer length (1000 sec. or 5 min. for example). This will allow the maximum light collection time, and the most signal and sensitivity.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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