

Secreted dual reporter assay with *Gaussia* luciferase and mCherry

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Abstract

This is a novel dual reporter assay for transfected tissue culture cells. It is based on the naturally secreted luciferase from *Gaussia princeps* as the main reporter protein and a secreted version of the red fluorescent protein mCherry as internal standard. After first measuring mCherry fluorescence in the medium, an enzyme buffer with coelenterazine as substrate is added to the same sample to trigger a glow-type luminescence of the luciferase. The simple and cheap assay can easily be adapted to a variety of experimental situations and should be an attractive alternative to the currently available commercial kits for dual reporter protein assays.

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Guidelines

Cell culture:

- Cells can be cultured in almost any medium and transfected by a wide variety of methods.
- Standard growth medium is replaced by Opti-MEM after transfection, for example the day after transfection. For a 6-well plate, use 1 ml of Opti-MEM.
- To measure the secreted reporters, harvest at least 100 µl of the supernatant (medium). Quick spin to remove floating cells or debris if necessary. For deferred measurements or long-term storage, the supernatants can be frozen and stored at -80°C.

Instrument and instrument settings:

- The assay can be adapted to a wide variety of multiwell and single tube readers.
- The following are the typical settings for a "Cytation 3 Cell Imaging Multi-Mode Reader" from Biotek, which can measure both fluorescence and luminescence:
 - Fluorescence: Endpoint, random, filter set 1 (excitation 570, emission 610), optics: top, gain: 120, light source: xenon flash, energy: high, read speed: normal with 100 msec delay, measurements/dapa point: 10, read height: 7 mm.
 - Luminescence: Endpoint, random, integration time: 0:01.00 (MM:SS.ss) (meaning one second), filter set 1 - emission: full; optics: top, gain: 120, read speed: normal with 100 msec delay, extended dynamic range, read height: 1 mm.

Before start

Required reagents:

- Opti-MEM ("Opti-MEM I Reduced Serum Medium, without Phenol Red") from Life Technologies. Note that this accounts for 90% of the price per sample.
- Coelenterazine is required as the substrate for *Gaussia princeps* luciferase (Gluc). We use native coelenterazine from BIOSYNTH (catalog number C-7001).
- Acidified methanol: add one drop of concentrated HCl to 10 ml of methanol.
- A 5 mM coelenterazine stock solution is prepared in acidified methanol and stored in aliquots (e.g. 10 µl) at -80°C (aliquots are stable for at least one year and can be freeze-thawed).
- Sodium iodide (NaI): prepare 1.5 M stock in water, adjust to more alkaline pH (e.g. pH 8.2), store at room temperature protected from light.
- Gluc buffer: 90 mM Tris-HCl pH 8.0, 15 mM NaCl, 0.3% Triton X-100, 75 mM NaI, 10 µM native coelenterazine. Prepare fresh from concentrated stocks on the day of use, keep at room temperature, protected from light, and add coelenterazine immediately before use.

Protocol

Step 1.

Transfer 100 µl of supernatant into 96-well dish (black flat bottom, non-binding surface polystyrene; e.g. Corning catalog number 3991).

Step 2.

Measure the mCherry fluorescence in a fluorometer (see Guidelines for detailed instrument settings).

Step 3.

Add 50 µl Gluc buffer per well to trigger the Gluc bioluminescence reaction. Since the luminescence gradually declines (to about 80% by 6 minutes; see Maguire et al., 2009, Anal. Chem. 81, 7102), it is important to work rapidly and beyond a certain number of wells, to use a multichannel pipet (or injector).

Step 4.

If you need to measure a more diluted sample, dilute supernatants separately with Opti-MEM and Opti-MEM complemented with 10% FBS for mCherry and Gluc, respectively.