

Manual for NanoFuel® FLASH Assay for Gaussia Luciferase:

Kit content:

Item	Volume	Storage
Lysisbuffer	50 ml	room temperature
Gaussia Dilution Buffer	50 ml	+4°C for up to 6 months or at room temperature for up to 2 months
Coelenterazine Dilution Buffer	50 ml	room temperature
50x Coelenterazine (CTZ) substrate	1 ml	optimally stored at -80°C, protect from light when handling outside of the freezer

The complete kit can also be stored long-term at -80°C.

Additional Materials Required:

- Pipettes and/or liquid handling equipment
- Luminometer or other light detecting instrument
 - Note:** this kit performs best using a Luminometer equipped with injector
- black 96- or 384-well microplates (e.g. Life Science Products, Cat. #MP8195-B)
- Optional: Gaussia expressing cells, Gaussia Luciferase protein as positive control (available at www.nanolight.com Cat. #321)

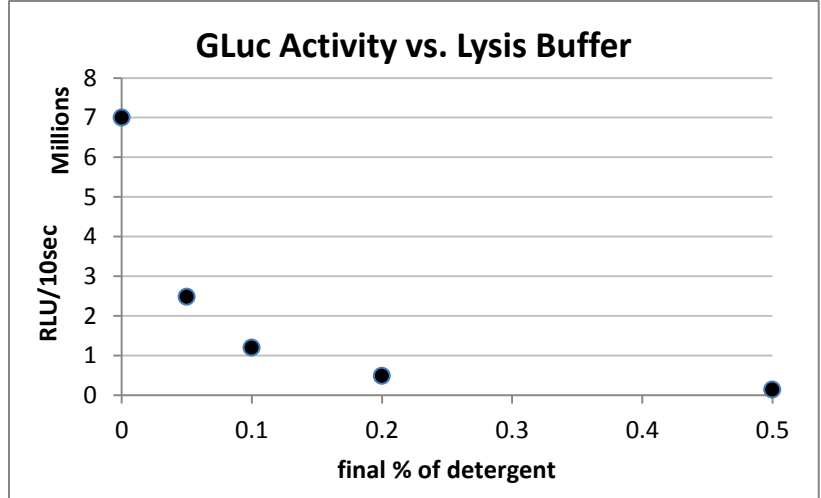
Protocol:

1) Cell lysis (optional)

- a. after cell transfection according to a standard mammalian cell protocol and sufficient incubation (16-72h) at 37°C, 5% CO₂ proceed to lysis
- b. lysis of cells is only necessary if Gaussia luciferase is not secreted
 1. pellet suspension cells by centrifugation (e.g. 400 x g for 5 min) and wash cells with PBS
 2. for adherent cells, aspirate the supernatant and wash cell monolayer carefully with PBS
 3. add 1 to 2 pellet volumes of the provided lysisbuffer or enough buffer to cover the cell monolayer (e.g. 25 µl for 96-well plates)
 4. mix by pipetting or shaking 15 min
 5. if the lysis was not complete please incubate for additional time

(Note: Coelenterazine is a very hydrophobic molecule and will pass through the cell membrane. The provided mild lysisbuffer will make the membrane more porous while only slightly reducing Gaussia Luciferase's activity. Dilution of the lysisbuffer with the Gaussia buffer will restore the activity

Depending on the cell-type, higher signals might be achieved by replacing the lysis detergent with a mechanical disruption (Dounce-homogenizer) of the cells in Gaussia Dilution Buffer.



2) Assay preparation

- dilute 1 part lysate with 1 part Gaussia Dilution Buffer (high amounts of detergents from the Lysisbuffer will reduce the activity of Gaussia Luciferase, see graph above)
- use 20-50 μ l of the diluted lysate in a black 96-well microtiter plate
- prepare Substrate-Buffer by adding 20 μ l of 50x CTZ-substrate to each 1 ml of CTZ-dilution buffer (e.g. add 100 μ l CTZ to 5 ml of CTZ dilution buffer)

Note:

- prepare this solution immediately before the measurement, Coelenterazine will degrade in the working solution over time, do not store
- please allow a few additional milliliters for priming the injector pump; please refer to your Luminometer manual for details
- high expression of Gaussia Luciferase might require higher dilutions in order to stay in the linear dynamic range of the luminometer

3) Measurement

- inject 50 μ l of substrate buffer into each well with a 2 second delay (time for a uniform mixing of the injected liquid) before integrating the signal for 5 to 10 seconds
- please perform the measurement within 30 min after addition of CTZ to CTZ dilution buffer

Important advice for usage of this product:

- Store Coelenterazine (50X) at -80°C and protect from light if handled outside. Briefly centrifuge tubes of Coelenterazine (50X) before use.
- Store CTZ dilution buffer and the resulting working solution at room temperature and protect from light. Before adding CTZ, the buffer must be at room temperature (20-25°C).
- Gaussia luciferase protein is very stable, nevertheless like every protein, it is susceptible to proteases, especially Aspartyl-Proteases. We recommend using Pepstatin A with a final concentration of 1 ug/ml as protease inhibitor if the cell lysate needs to be stored.
- Gaussia luciferase will keep its activity over multiple freeze/thaw cycles.
- Do not expose reagents to excessive heat or light during storage and incubation (do NOT thaw reagents using hot water or heating blocks)
- Do not mix reagents from different lots. Discard unused working solutions after assay completion.
- Only prepare as much CTZ working solution as needed.
- Follow good laboratory practice in handling reagents and machines.

Kit containing buffers were designed to work with the following Gaussia Luciferase Proteins:

- wild-type GLuc
- GLuc-M2 (M43L, M110L)
- GLuc-8990 (F72W, I73L)

This product is covered by US Patents #7,109,315 6,232,107, 6,436,682, 6,780,974 and World Patent WO1999/049019.