NanoFuel® FLASH Assay for Gaussia Luciferase, Cat. #319

Kit content:

- 50 ml Lysisbuffer
- 50 ml Gaussia Dilution Buffer
- 50 ml Coelenterazine Dilution Buffer
- 1 ml 50x Coelenterazine (CTZ) substrate

Storage:

The NanoFuel FLASH Assay Kit is shipped with ice bricks. Upon receipt, please store the kit at -80°C or individual components at indicated temperatures.

Upon thawing (at room temperature) please aliquot and/or store buffers at +4°C for up to 6 months or at room temperature for up to 2 months. CTZ dilution buffer **must** be at room temperature (20-25°C) before adding the 50x CTZ substrate.

The Coelenterazine substrate should be optimally stored at -80°C. It will not freeze and can conveniently be used directly out of the freezer. Please protect from light if handled outside the freezer. Coelenterazine will be stable for at least one year if stored at -80°C.

Quick Protocol:

1. Lysis (optional)
    a. pellet cells by centrifugation (e.g. 400 x g for 5 min)
    b. remove supernatant and lyse cell by adding the one pellet volume of Lysisbuffer
    c. mix by pipetting, incubate for 15 min

2. Assay preparation
    a. dilute 1 part lysate with 1 part Gaussia Dilution Buffer (high amounts of detergents from the Lysisbuffer will reduce the activity of Gaussia Luciferase)
    b. use 20-50 µl of the diluted lysate in a black 96-well microtiter plate
    c. prepare Substrate-Buffer by adding 20 µl of 50x CTZ-substrate to each ml of CTZ-dilution buffer (discard after use, do not store)

3. Measurement
    a. inject 50 µl of substrate buffer into each well with a 2 second delay before integrating the signal for 10 seconds

For a more comprehensive protocol please visit our website at www.nanolight.com.

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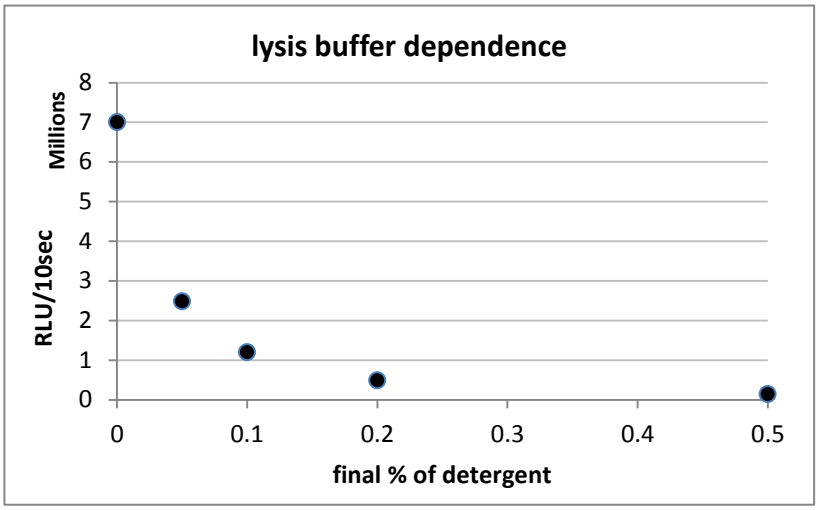
Tips for use of NanoFuel® FLASH Assay

Lysisbuffer:

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 (please see graph below).



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.

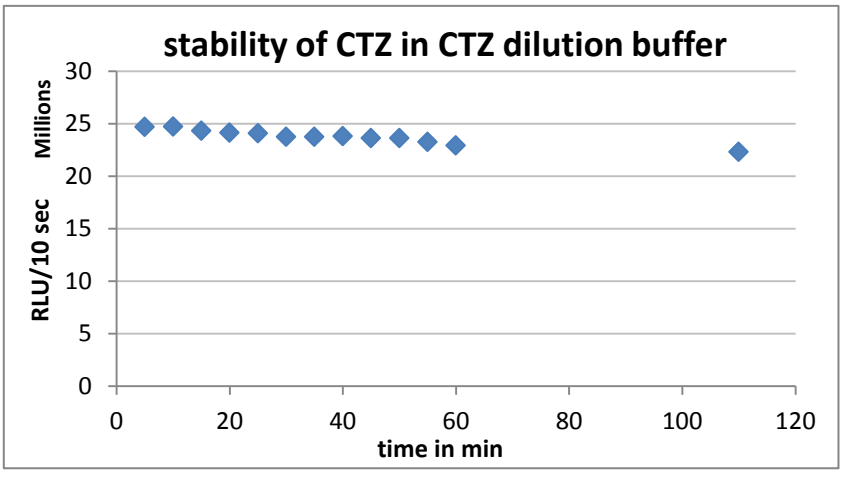
Gaussia dilution buffer:

Gaussia dilution buffer provides the right conditions (pH, salt) for optimal performance of Gaussia Luciferase. It also stabilizes the protein for storage at 4°C. The protein can also be stored frozen. Multiple freeze-thaw cycles will not reduce its activity.

CAUTION: Gaussia is especially susceptible to Aspartyl-Proteases, thus we recommend using Pepstatin A with a final conc. of 1 ug/ml as protease inhibitor if the lysate needs to be stored for an extended period of time.

Coelenterazine dilution buffer:

Please prepare the substrate buffer fresh before your bioluminescent measurement. We are providing a 50x concentrated substrate that will be diluted to 1x with CTZ dilution buffer. This buffer is designed to keep CTZ active during the time of measurement (up to 2 hours).



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

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