

Manual for NanoFuel® GLOW Assay for Oplophorus Luciferases like NanoLuc® or NanoKAZ

Materials supplied:

- 50 ml NanoFuel® GLOW Reagent for Oplophorus Luciferase
- 1 ml 50x Oplo-GLOW Substrate for Oplophorus Luciferase

Storage:

Long term at -20°C. Thaw at RT.
 Store at 4°C up to 9 months.
 Always store at -80°C.
 Solution will be liquid at this temperature.

Instructions:

1. Thaw NanoFuel® Assay Reagent Buffer at room temperature (RT). Do not heat. Shake the bottle before use.
2. Add required amount into a reagent tube (e.g. 5 ml)
3. Add 20 µl of substrate to each 1 ml of buffer (e.g. 100 µl substrate to 5 ml buffer , aspirate the viscous substrate solution slowly)
4. Mix by inverting or pipetting. Keep at RT for immediate use or store at 4°C for use up to 24 hours later. We recommend making the buffer/substrate solution fresh before every use for consistent results.
5. Add 1 to 2 volumes (e.g. 50-100 µl) of the buffer+substrate to one volume of your cells / cell supernatant (e.g. 50 µl). All solutions should be equilibrated at RT.
6. Mix and wait 5 minutes before measuring.
7. Integrate the signal for 5 to 10 seconds to obtain a more consistent reading in your luminometer.

Stability in high-throughput screens (HTS):

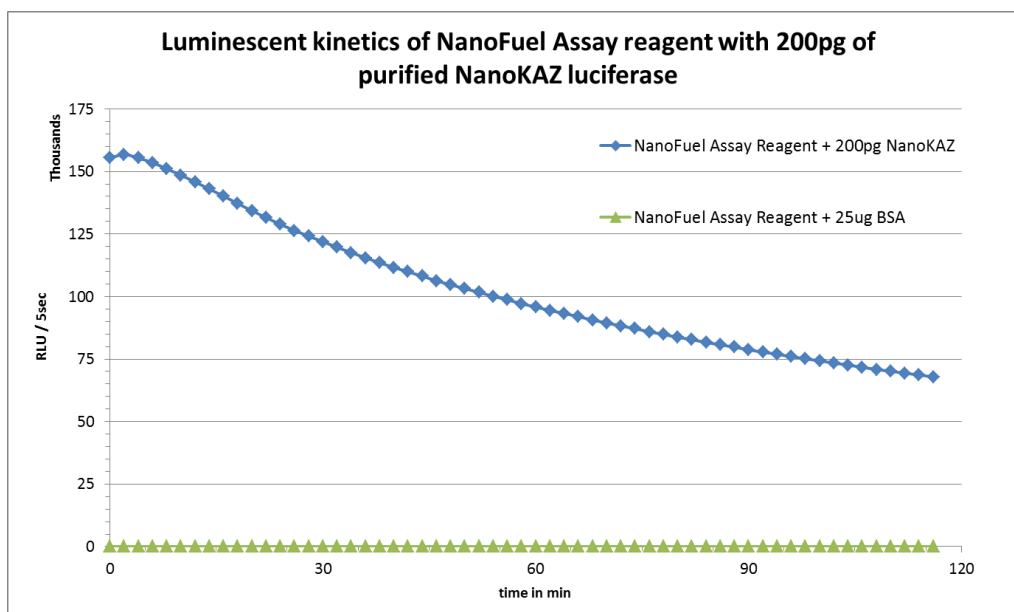


Fig. above: 50 µl NanoFuel® GLOW Reagent were added to 200pg of purified NanoKAZ luciferase (in PBS) and measured for 2 hours. The signal represents relative light units (RLU) collected and integrated over a period of 5 seconds. 25 µg of BSA was used as a control.

Practical Notes:

1. NanoFuel® Assay Reagent will generate Bioluminescence with all versions of the Oplophorus Luciferase including but not limited to:

Oplophorus Luciferase (wildtype, 196 aa, 19.5 kDa) GI:10336559

MAYSTLFI IALTAVVTQASSTQKSNLFTFLADFGVDWQQTAGYNQDQVLEQGGSSLFQALGVSVTPIQKVLS
GENGLKADIVHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIILHYGTLVIDGVTNPMIDYFGRPYPGIAVFD
GKQITVTGTLWNGNKIYDERLINPDGSLFRVTINGVTGWRLCENILA

NanoKAZ (171 aa, 19.1 kDa) GI:525342150

MVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIVHVIIPYEGLSGDQMGQ
IEKIFKVVYPVDDHHFKVILHYGTLVIDGVTNPMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPD
GSLFRVTINGVTGWRLCERILA

NanoLuc® (171 aa, 19.1 kDa) GI:386649645

MVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIVHVIIPYEGLSGDQMGQ
IEKIFKVVYPVDDHHFKVILHYGTLVIDGVTNPMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPD
GSLFRVTINGVTGWRLCERILA

2. High amounts of Luciferase with cause a faster depletion of the substrate thus reducing the half-life of the bioluminescent signal. Try to dilute your sample or reduce the number of transformed cells per well in order to compensate for the high luciferase expression.
3. The bioluminescent signal has a half-life of approx. 2 hours.
4. Luminometers have a high dynamic range. This range is not completely linear. Please ensure that you measure your signal within the linear range of your instrument.
 - a) establish a standard curve
 - b) adjust the integration time for your experimental setup
5. NanoFuel® Assay Reagent was especially designed to reduce the background caused mostly by serum albumin. In the rare case that the background signal is too high, dilute the lysate or wash the cells in serum free media.
6. Always equilibrate the buffer to room temperature before adding the substrate.
7. This buffer contains a detergent that will lyse the cells and give a signal with intracellular expressed luciferase as well.

NanoLuc® is a registered trademark of Promega Corp.