

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

Materials supplied:

- 50 ml NanoFuel® FLASH Reagent for Oplophorus Luciferase
- 1 ml 50x NLuc-FLASH Substrate

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® FLASH 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. We recommend making the buffer/substrate solution fresh before every use for consistent results.
5. Connect the injector tube from your luminometer to the tube containing the FLASH working solution. Prime the tubing and follow the manufacturer's instructions of the luminometer.
6. 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 to RT.
7. The integration time depends on the type of assay you want to perform. We recommend a 2 sec. delay and a 10 sec. integration as a starting point. Please see the graph below as a guideline for the luminescent signal.

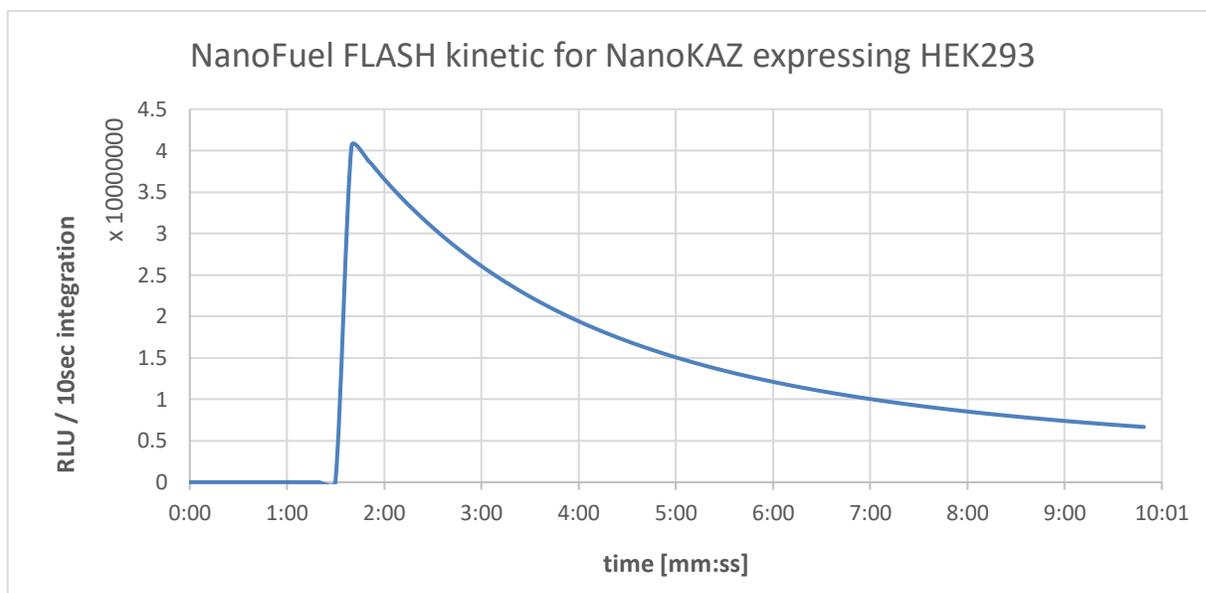


Fig. above: 50 µl NanoFuel® FLASH Reagent was added to 50 µl transfected HEK293 cells in media after 1:30 min. The signal represents relative light units (RLU) integrated over a period of 10 seconds for a total collection time of 10 minutes.

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 IALTAVVTQASSTQKSNLTFTLADFVGDWQQTAGYNQDQVLEQGGSSLFQALGVSVTPIQKVLS
GENGLKADIVHVIIPYEGLSGFQMGLIEMIFKVVPVDDHHFKIILHYGTLVIDGVTPNMIDYFGRPYPGIAVFD
GKQITVTGTLWNGNKIYDERLINPDGSLFRVTINGVTGWRLCENILA

NanoKAZ (171 aa, 19.1 kDa) GI:525342150

MVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIVHVIIPYEGLSGDQMGQ
IEKIFKVVPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPD
GSLFRVTINGVTGWRLCERILA

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

MVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIVHVIIPYEGLSGDQMGQ
IEKIFKVVPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPD
GSLFRVTINGVTGWRLCERILA

2. High amounts of Luciferase will 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. Luminometers have a high dynamic range. Nevertheless, 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 (for example with purified NanoKAZ protein, **Cat. #381**)
 - b) adjust the integration time for your experimental setup
4. NanoFuel® Assay Reagents are specially designed to reduce the background caused mostly by serum albumin (BSA, FCS). In the rare case of a high background signal, dilute the lysate or wash the cells in serum free media.
5. Always equilibrate the buffer to room temperature before adding the substrate.
6. This buffer does not contain a detergent that will lyse the cells. Please order our universal lysis buffer (**Cat. #333**) for intracellular expressed Oplophorus luciferase.

NanoLuc® is a registered trademark of Promega Corp.