



# NanoFuel® GLOW Assay for Gaussia Luciferase

## #320 NanoFuel GLOW Assay Kit content:

- 50 ml NanoFuel® GLOW Reagent for **Gaussia Luciferase**
- 1 ml **50x** Coelenterazine Substrate for GLOW assay

## Storage:

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

Upon thawing (do not heat) 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 50x CTZ substrate.

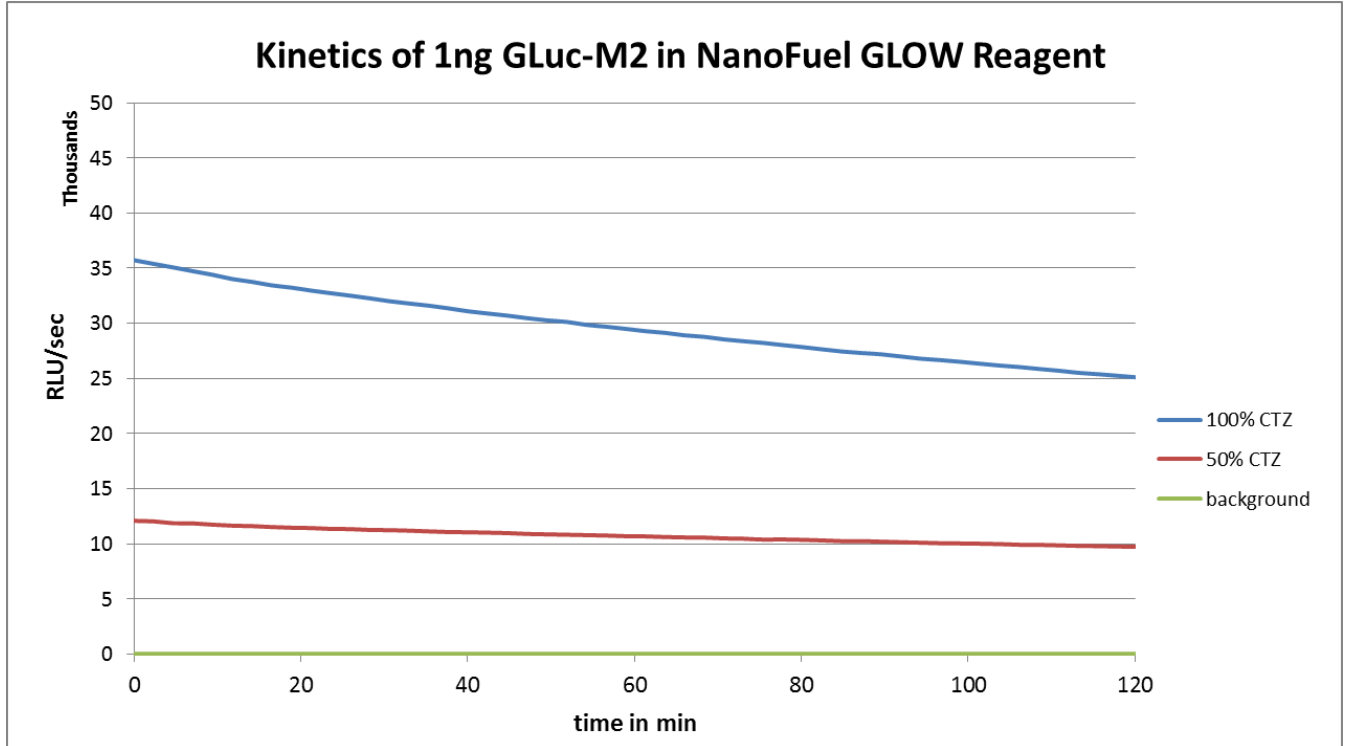
The Coelenterazine substrate should be optimally stored at -80°C. It will not freeze and can conveniently be used directly out of the freezer. Please protect from light if handled outside the freezer. Coelenterazine will be stable for at least one year if stored at -80°C.

## Quick protocol:

1. Allow the buffer and cell culture supernatant to equilibrate to room temperature.
2. Prepare NanoFuel® GLOW working solution by adding 20 ul of 50x substrate to each 1 ml of buffer
3. add one volume of this solution (e.g. 50 ul) to one volume of cells/ cell supernatant (e.g 50 ul) into a black 96-well or 384-well plate
4. wait 5 min before measuring the luminescence (the luminescence will decay in nearly a linear fashion with a half-life of approx. 3 hours)

## Practical advice:

- this buffer will perforate cells to measure internal GLuc expression
- in order to just measure secreted GLuc please remove cells by centrifugation
- measure background readings first to prevent 'bleed-over' effects
- this buffer is designed to reduce the background, if the background is still too high please dilute out the FCS in your media with PBS or a similar buffer
- integrate your signal for 5 to 10 sec. to get a more stable reading
- determine the linear range of your Luminometer by measuring a dilution series of Gaussia luciferase protein (available at [www.nanolight.com](http://www.nanolight.com) , Cat. #321)
- use Gaussia-M2 mutant sequence for best performance in glow kinetic assays (please contact us for more info)
- do not store the working solution



**Fig.1:** Dependence of signal stability and luminescent intensity on Coelenterazine amount. 100% CTZ is using the supplied substrate as 50x solution. 50% CTZ refers to a usage as 100x substrate.

- using only half the amount of CTZ (as 100x) will result in a longer lasting glow with a lower signal, please adjust your assay accordingly
- dilute your sample if you are out of the linear range of your Luminometer or if the luminescent signal decays too rapidly