



HOW TO USE “Inject-A-Lume”

Background: Highly pure Coelenterazine and its analogs form crystalline structures that make them extremely difficult to dissolve using physiological less toxic solvents.

We can provide an empirically designed solvent called “**Fuel-Inject**” within our “Inject-A-Lume” kit. It is able to dissolve extremely pure (99%+) Coelenterazine, h-Coelenterazine, Coelenterazine-F, and our other specially packaged sterile Coelenterazine analogs, in a relatively safe and effective injectable solution.

Injectable Nanofuel™: All injectable NanoFuel™ products contain 0.5 milligram amount of NanoFuel™ freeze-dried on an inner glass vial as a thin film lining for easier dissolution. For longer shelf-life all injectable NanoFuels™ are packed under Argon and packaged with an oxygen absorber and silica gel desiccant packs to prevent oxidation. Open immediately before use.

Our special injection-vials have a low retention volume but therefore only a 300 µl maximal volume capacity. You may unscrew the top if desired for access using larger needles.

Instructions:

1. Using any 0.5 to 1.0 ml syringe equipped with a 23 or smaller gauge needle, draw up **150µl** of warm (35-50°C) ‘Fuel-Inject’ diluent.
2. Inject **150µl of “Fuel-Inject”** into the NanoFuel™ vial. Aspirate up and down using the needle and syringe or vortex briefly, observe for a completely clear solution, if the solution is not completely clear, warm the vial under hot water or in a heating block at 60-80°C for few minutes and inspect for complete clarity.
3. Draw up 15 to 75 µl (see table below) of dissolved NanoFuel™ (3.33 mg/ml) with Insulin syringe (e.g. BD cat. # 328430) syringe for injection via tail vein. Inspect for, and remove any air bubbles in the syringe and flush the needle. The advantage of using Insulin syringes is their very low (<2µl) holdup volume! **Inject slowly.**

We recommend using the following amounts of your 3.33 mg/ml NanoFuel™ solution for injecting a mouse (25 gram body weight):

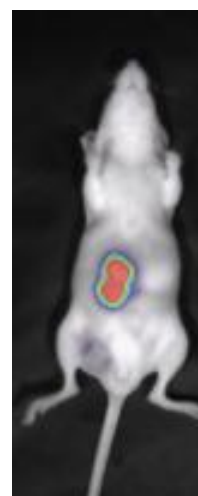
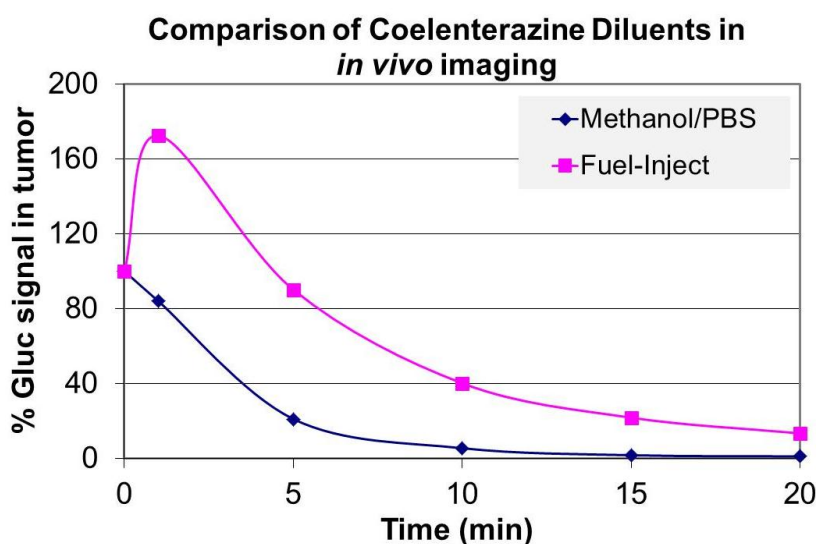
<u>Substrate Amount Desired</u>	<u>Injection Volume</u>
50 µg	15 µl
100 µg	30 µl
200 µg	60 µl
250 µg	75 µl

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Other Information: “Fuel-Inject” diluent was designed to maximize the concentration of Coelenterazine and minimize volume injected for all our currently available Coelenterazines.

Bio-distribution: “Inject-a-Lume” was tested in mouse tail vein injection for which this product was designed. In comparison to conventional Methanol/PBS dissolved CTZ ‘Fuel-Inject’ will improve your *in vivo* imaging results.

Data presented below was kindly provided by Dr. Bakhos Tannous*
<http://www.ncbi.nlm.nih.gov/pubmed?term=tannous AND gaussia>:



*Gaussia Luciferase is many times brighter than other luciferases, (has a much higher Km), you will have to use more Coelenterazine to appreciate its potential; (native Coelenterazine is the only substrate that will work with Gaussia Luciferase). We recommend using 100-200 µg in a 25 gram mouse, more for higher signal. **Do not use more than 70-80 µl ‘Fuel-Inject’ per 25 gram mouse.** Raise the concentration of the Coelenterazine solution for injection of higher substrate amounts.