# 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-live all injectable NanoFuels™ are packed under Argon to prevent oxidation. Store vials at -80°C upon arrival and let them get to room temperature before use.

Our special injection-vials have a low retention volume with 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 (20-40°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°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 into the peritoneal cavity. 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):

| Desired Substrate Amount | Injection Volume |
|---|---|
| 50 µg | 15 µl |
| 100 µg | 30 µl |
| 200 µg | 60 µl |
| 250 µg | 75 µl |

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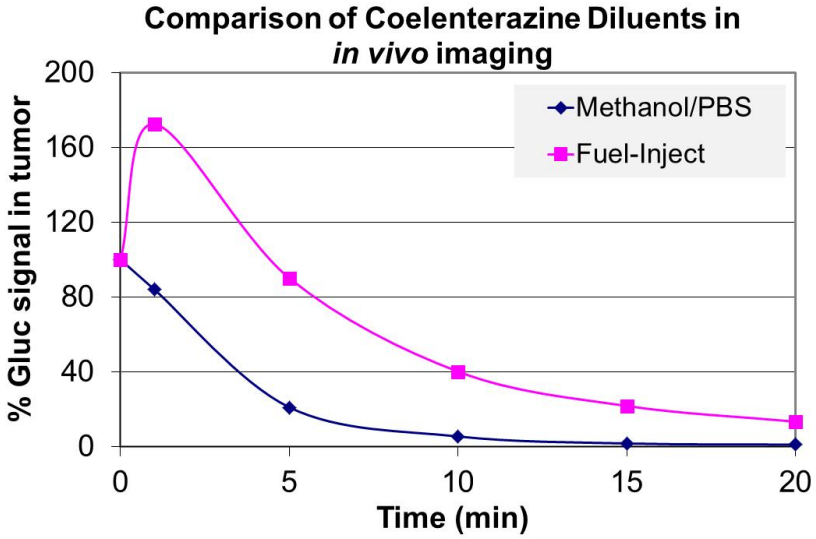
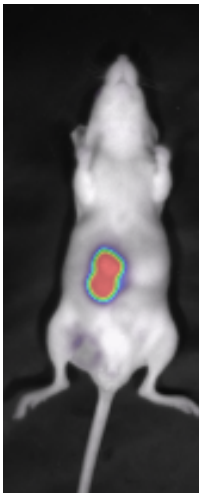
**Other Information:** "Fuel-Inject" diluent was designed to maximize the concentration of Coelenterazine and minimize volume injected for all our currently available Coelenterazines.

**Bio-distribution:** "Inject-a-Lume" was tested in mouse tail vein injection but will also work for intra peritoneal injections. 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 $K_m$), 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.

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