



Creation Date: 6/24/2014 Revision Date: 3/6/2019

Luciferin FAQ

Does your Luciferin work in vivo?

Answer: YES! Many of our customers use our Luciferin in animals. Our Luciferin has been cited in over 2000 peer-reviewed journal articles. **See below** for an example of <u>GoldBio</u> <u>Luciferin</u> used in an *in vivo* luciferase mouse assay:



Does Luciferin purity matter?

Answer: YES! Goldbio Luciferin is **99.7%** to **99.8%** pure, the highest purity Luciferin commercially available. Other vendors would have you believe that 98% pure is "good enough". However, this doesn't stand up to rigorous evaluation. For instance, consider a Luciferin product which is <u>only 99% pure</u> (which therefore has 1% contamination inherent in the product). If 1 gram of the less pure Luciferin is dissolved in 25 ml of buffer (a standard dilution for in vivo assays), there would be 0.01 g of contaminant in the buffer (a

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concentration of 0.4 g per liter)! If the contaminant in this example had a molecular weight of 1000 g/mol, the concentration of the **contaminant in solution would be 400µM**, which is above the concentration necessary to inhibit certain enzymes and biological processes in a cell and has the potential to **skew results** and **harm your animals**.

Is there a difference between Beetle Luciferin and Firefly Luciferin?

Answer: No, there is no difference between the Beetle and Firefly Luciferin. For structural information, please see "Beetle vs. Firefly Luciferin" in the Additional Information section.

How do you dissolve Luciferin? How stable is Luciferin?

Answer: The literature and some customers tell us that they dissolve Luciferin in water, and then freeze aliquots at -80°C, with no significant deleterious effects. Potassium salt is usually soluble at 60 mg/ml while Sodium salt is soluble up to 100 mg/ml (**Free acid is not soluble in H₂O**, but is soluble in methanol at 10 mg/ml or in DMSO at 50 mg/ml). However, there are conflicting reports concerning the stability of dissolved Luciferin which may be caused by the amount of available oxygen in the water. Dissolve Luciferin in degassed H₂O for best storage results.

For the most sensitive experiments, however, we strongly recommend that you use freshly made reactants in order to reduce the variables in your experiment (for example, low concentration of enzyme or sub optimal temperature or sub optimal salt concentration might require high substrate concentrations to drive the reaction to completion).

Is there a difference between Sodium and Potassium Luciferin?

Answer: We do not see an applicable difference between the sodium and potassium salts of Luciferin. There are small physical characteristic differences, such as sodium Luciferin is somewhat more granular and is more soluble than potassium Luciferin. From literature, potassium Luciferin is cited approximately 3 times more often than sodium Luciferin, and most researchers seem to prefer potassium Luciferin *in vivo*, but either Luciferin salt will work equally well.

Is there a difference between Sodium/Potassium Luciferin and Free Acid Luciferin?

Answer: The Free Acid form of Luciferin will not dissolve in water unless a dilute base such as NaOH or KOH is added to adjust the pH. However, it is soluble in Methanol at 10 mg/ml or in DMSO at 50 mg/ml. However, the Potassium or Sodium Salt of Luciferin is more convenient for experiments, *particularly for in vivo imaging*, since either will readily dissolve in water or



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buffer, which is less toxic in those systems.

Why do the protocols say to dissolve Sodium/Potassium Luciferin in Ca²⁺ or Mg²⁺ free PBS? Can other saline solutions be used to dissolve the Luciferin?

Answer: PBS, or phosphate buffered saline, and Dulbecco's phosphate buffered saline (DPBS), are buffered salines which are often used in biological studies and most typically used in research involving cells. PBS/DPBS is an isotonic buffer (i.e. compatible with the human body). These buffers are designed to provide and preserve a stable pH of 7.2-7.6. There is no difference between PBS and DPBS, although DPBS is often made without calcium or magnesium. Calcium and magnesium solutions may restrain the activity of trypsin. Additionally, Mg²⁺ is a crucial ingredient in the catalysis of Luciferin to Oxyluciferin (and Ca²⁺ is similarly involved in the coelenterazine catalysis). Other saline solutions may be used to dissolve Luciferin as long as the cation presence does not interfere with the results of the experiment or create off target effects.