Manual for h-Coelenterazine

Cat.# 301 h-Coelenterazine CAS# 50909-86-9 MW 407.46

Alternative names: Renilla Luciferin, 2-Deoxycoelenterazine, Coelenterazine-h

General Notes: h-Coelenterazine (h-CTZ) is the dehydroxy derivative of native Coelenterazine and can be used to increase the initial luminous intensity of Aequorin up to 20-fold, thus becoming a more sensitive Ca^{2+} sensor.
Dr O. Shimomura (Biochem. J. 1989 Aug 1;261(3); 913-920) was able to show that h-Coelenterazine produced 10 times more initial light intensity but the total number of photons produced was 82% overall. Essentially the initial light output was compressed and 10 times the flash intensity was noted over a moderately shorter rise time.
h-Coelenterazine is also used as substrate for Renilla Luciferase, creating a lower initial intensity but a longer lasting kinetic.
h-Coelenterazine should not be used with Gaussia Luciferase, it will require native Coelenterazine (Cat.#303).

Storage and Storage Life: It is best stored as completely dry powder under Argon in air-tight O-ring plastic tubes at -20°C or for longer storage at -80°C, protected from light. Oxygen and moisture will lead to auto-oxidation of CTZ over time, reducing its overall activity.

Dissolving h-Coelenterazine: We recommend using our specifically developed NanoFuel Solvent (Cat. #399) for maximum solubility and shelf-life. Adding 500 µl to 500 µg of lyophilized h-Coelenterazine will result in a 1 mg/ml solution that can be stored at -20°C or below for at least one year without any notable degradation.
As an alternative you may use Methanol to dissolve h-Coelenterazine. To prevent oxidation, it is recommended to acidify and degas the alcohol prior to addition.

Dilution and luminescent assaying: We recommend to prepare the working solution fresh every time before a luminometer assay. In general, a 100 µM h-Coelenterazine solution will work with most assays. Use 407.5 µl of the 1 mg/ml stock solution and dilute in 10 ml of your buffer of choice (e.g. PBS) to get a 100 µM solution. All solutions should be at room temperature.
When performing a plate reader assay and you want to compare relative light units (RLU) results between the first well and last well in a 96 plate, please note that h-CTZ will continuously oxidize over time in aqueous solutions (see graph, performed with two widely used buffers).