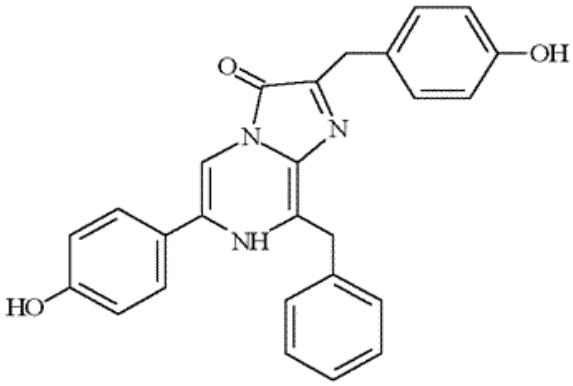
Post Office Box 2746 Pinetop, Arizona 85935
163 W. White Mountain Blvd. Pinetop, Az 85935
Telephone (928) 367-1200 Fax (928) 367-1205
www.nanolight.com

How to use Coelenterazine and its analogs:
Catalog Numbers NF-CTZ-FB & NF-BCTZ-FB

Native Coelenterazine (nCTZ) FW= 423.5 ((2-(p-Hydroxybenzyl)-6-(p-hydroxyphenyl)-8-benzyl-imidazol[1,2-a]pyrazin-3-(7H)-one



Benzyl Coelenterazine (hCTZ) FW=407.5 (hCTZ is then dehydroxy deriviative the R1 right handed Phenyl group –OH is replaced by –H)

General Notes:

Once mixed, coelenterazine (CTZ) solutions oxidize and gradually decay to their coelenteramide oxidation product. Dry coelenterazine compounds are only sparingly soluble in aqueous solutions, and must be dissolved in alcohols, propylene glycol, or straight B-mecaptoethanol prior to making aqueous buffer solutions. Mecaptoethanol solutions may inhibit or destroy luciferase activity buit are fine for Aquorin and Obelin (calcium activated photoprotein) applications.

If DMSO is used in conjunction with CTZ you must mix and use immediately, DMSO will decompose the CTZ and will not give uniform repeatable results.

A: In Vitro Use

Add to PBS buffers that are close to neutral, Aqueous buffers with pH 6.6-7.2 work best at avoiding precipitates, but the buffer chosen should be used in conjunction with the enzyme system. The optimal activity of most marine luciferases occur in buffers that are close to Sea Water in composition (500mM NaCl pH 7.8).

If you are using multi-well plates and have to assay each well over a given amount of time, you should allow the aqueous solution of CTZ to stand at room temperature for 25-30 minutes prior to plate reading. IF freshly mixed CTZ solutions are used, the initial activity read in the first plates will be quite a bit higher then the last plates due to the auto-oxidation of CTZ in water, this will be minimal if the 25-30 minute stabilization period is observed.

Specially designed high throughput screening assay type buffers that stabilize and prolong the CTZ signal for up to 30 minutes are available please enquire (info@prolume.com), since these are new products and not available for general release as of March 2005.

B: In Vivo studies

For Renilla luciferase 25-100 micrograms/mouse via IP or IV injection will give good results. For Gaussia luciferase, higher concentrations should be used due to the increased turnover activity of Gaussia luciferase. Thought should be given to using ethanolic rather than methanolic mixtures to live animals, and propylene glycol suspensions of CTZ, which may take longer to dissolve, it works quite well at solubilizing CTZ and will not cause venous injury that the alcholic suspensions incur; recommended in the case or repeated studies over many days.

C. General Resuspension and Storage Solution:

To 10ml of 100% Methanol or Ethanol add 200ul of 3N or 6N HCl mix well. Take 0.25-1ml of the acidified alcohol and weigh out up to 10mgs of CTZ/ml of alcohol. Usually 1mg/ml is used, and it should go into suspension quite rapidly. 10mgs/ml is about the most concentrated and a slight precipitate may form at this concentration.

Store at –20C or better –70C. While not as good as freshly mixed CTZ, this should be good for 1-3 weeks. For accurate reproducible comparative data, freshly mixed is recommended for best results. Always protect any CTZ from light.