

Cat.# 340 Coelenterazine 400a CAS# 70217-82-2 MW = 391.5

**Alternative names:** Coelenterazine 400a, 1-bisdeoxycoelenterazine, di-dehydrocoelenterazine, DeepBlueC™

**General Notes:** Coelenterazine 400a (CTZ-400a), named after the maximum in emission wavelength from its oxidation by *Renilla reniformis* and *Renilla mulleri* Luciferase. In these luciferases CTZ-400a is causing a blue-shift in wavelength compared to the 475 nm emission peak using native Coelenterazine.

CTZ-400a is primarily used in bioluminescent resonant energy transfer (BRET) as occurs when Renilla luciferase is in close proximity to a Green Fluorescent Protein (GFP).

This energy transfer property is exploited to design proximity assays in which protein-protein interactions as in GPCR mediated (and other) drug cell signaling interactions occurs, and therefore can be used as a drug development and screening tool.

**Storage and Shelf-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 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 Coelenterazine 400a 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 Coelenterazine 400a. 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 Coelenterazine 400a solution will work with most assays. Use 391.5 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 96 plate, please note that CTZ will continuously oxidize over time in aqueous solutions.

### Alternative Products:

Prolume/NanoLight developed a patented substrate that is emitting light at 407 nm at a 10-fold intensity compared to CTZ-400a. ProlumePurple (PP-CTZ, Cat.# 369), can be used with Renilla Luciferase and its mutated versions (like RLuc8, RLuc3).

