# Coelenterazine *ip*

**Luminescent calcium indicator and Renilla luciferase substrate**

PromoKine

## Instruction Manual

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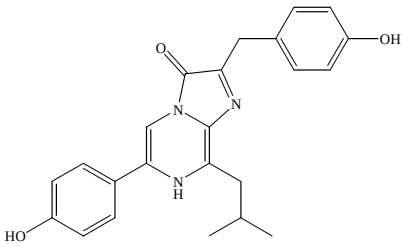
| **Catalog Number** | PK-CA707-10116 |
| **Description** | Coelenterazine *ip* is a synthetic derivative of coelenterazine. The luminescence intensity of its aequorin complex is almost 50 times higher than that of the aequorin formed from native coelenterazine while its response time to calcium is much slower than the latter.<br>Coelenterazine and its analogs can be used as luminescent calcium indicator and for luciferase assays (e.g. with the Renilla luciferase reporter gene). Other uses of coelenterazine include chemiluminescent detection of superoxide anion and peroxynitrite in cells or tissues. (Find more information under „Applications“). |
| **Quantity** | 50 µg |
| **Excitation / Emission Maxima** | λex\λem = 430/441 nm;<br>Extinction coefficient: 7,000 (in MeOH) |
| **Molecular Structure** |  |
| **Molecular Weight / Molecular Formula** | 389.45 Da; $C_{23}H_{23}N_3O_3$ |
| **Purity** | >98% (as determined by HPLC) |
| **Appearance / Formulation / Solubility** | Yellow solid. Coelenterazine and the derivatives can be reconstituted by dissolving in methanol or ethanol. **Do not dissolve in dimethyl-sulfoxide (DMSO)** as coelenterazine *ip* may be unstable in this solvent. Low solubility in water. |
| **Storage & Stability** | Store at –20°C. Store in tightly sealed vial. Protect from light.<br>Solution is susceptible to oxidation by air. For best results, keep solution from light and store at <-70°C under nitrogen or argon. Keep solid at -20°C or -70°C and protect from light under nitrogen or argon for long-term storage. Keep calcium free when stored in solution (avoid using glass container). |
| **References** | 1) *Biochem. J.* **261**, 913(1989)<br>2) *Cell Calcium* **12**, 635(1991)<br>3) *Cell Calcium*, **14**, 373 (1993);<br>4) *Mol Imaging*, **3** (1), 43(2004 Jan) |
| **Caution** | Potentially harmful. Avoid prolonged or repeated exposure. Avoid getting in eyes, on skin, or on clothing. Wash thoroughly after handling. If eye or skin contact occurs, wash affected areas with plenty of water for 15 minutes and seek medical advice. In case of inhaling or swallowing, move individual to fresh air and seek medical advice immediately. |

## Applications

Coelenterazine is the natural substrate for Renilla luciferase, an enzyme derived from sea pansy, which catalyzes coelenterazine oxidation by oxygen to produce light. Luciferase is used as a reporter gene for luminescence based assays. However, over a dozen of coelenterazine analogs have been synthesized, many of which are now commercially available from PromoKine. These coelenterazine analogs can function as substrates for Renilla luciferase, and have different properties in terms of emission wavelength, cell membrane permeability and quantum efficiency. Coelenterazine also emits light from enzyme-independent oxidation, a process known as autoluminescence. The autoluminescence is enhanced by superoxide anion and peroxynitrite in cells and tissues. Thus, coelenterazine is also used for chemiluminescent detection of superoxide anions and peroxynitrite in cells and tissues.

PromoKine offers high purity native coelenterazine and a number of coelenterazine analogs. Table 1 summarizes the luminescent properties of coelenterazine derivatives with Renilla luciferase. As the table shows, both native coelenterazine and coelenterazine *e* are good substrates for Renilla luciferase. In addition to consideration of quantum yields, emission wavelength may become important when Renilla luciferase in combination with a fluorescent protein such as GFP is used in bioluminescent resonance energy transfer (BRET), an important application for the studies of protein-protein interactions.

Coelenterazine and its analogs also bind to the jellyfish protein apoaequorin to form aequorin, a calcium-sensitive bioluminescent protein that can be used for bioluminescent detection of calcium with high sensitivity and a large dynamic range and that has been used extensively as a microinjectable calcium indicator in cells. Coelenterazine is membrane permeable, and can be used to facilitate the reassembling of the aequorin complex *in vivo*. Coelenterazine is oxidized and illuminates blue light at 466 nm when $Ca^{2+}$ binds to the complex. The luminescence intensity is correlated to the $Ca^{2+}$ concentration. Compared with fluorescent calcium indicators, aequorin has several advantages in monitoring intracellular calcium. One major advantage is that the aequorin complex can detect a broad range of calcium concentrations, from ~0.1 µM to >100 µM. Another advantage is that the aequorin complex is stably retained inside cells, making it possible to follow calcium concentration changes for hours to days. Table 2 lists the luminescent properties of coelenterazine analogs in complex with apoaequorin.

Table 1. Luminescent Properties of Coelenterazine Analogs with Renilla Luciferase*

| Analog | λem(nm) | Total Light (%) | Initial Intensity (%) |
|---|---|---|---|
| native | 475 | 100 | 45 |
| 400a | 400 | | |
| *cp* | 470 | 23 | 135 |
| *e* | 418, 475 | 137 | 900 |
| *f* | 473 | 28 | 45 |
| *h* | 475 | 41 | 135 |
| *n* | 475 | 47 | 900 |

Table 2. Luminescent Properties of Coelenterazine Analogs with Apoaequorin**

| Analog | λem(nm) | Relative luminescence capacity | Relative intensity | Half-rise time (s) |
|---|---|---|---|---|
| native | 465 | 1.0 | 1.00 | 0.4-0.8 |
| *cp* | 442 | 0.95 | 15 | 0.15-0.3 |
| *e* | 405, 465 | 0.50 | 4 | 0.15-0.3 |
| *f* | 473 | 0.80 | 18 | 0.4-0.8 |
| *fcp* | 452 | 0.57 | 135 | 0.4-0.8 |
| *h* | 475 | 0.82 | 10 | 0.4-0.8 |
| *hcp* | 444 | 0.67 | 190 | 0.15-0.3 |
| *i* | 476 | 0.70 | 0.03 | 8 |
| *ip* | 441 | 0.54 | 47 | 1 |
| *n* | 467 | 0.26 | 0.01 | 5 |

* Data from Biochem. Biophys. Res. Commun. 233, 349 (1997).
** Data from Biochem. J. 261, 913 (1989).

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