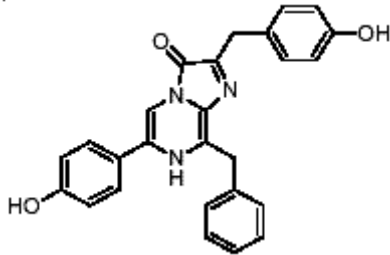


Coelenterazine (native)

Luminescent calcium indicator and Renilla luciferase substrate

Instruction Manual

Catalog Number	PK-CA707-10110
Description	<p>Coelenterazine (native form) is a luminescent enzyme substrate for apoaequorin and Renilla luciferase. The aequorin complex has been used as an excellent calcium indicator that can measure a broad concentration range of calcium from ~0.1 μM to >100 μM. Renilla luciferase, encoded by the luc gene, has been widely used as a reporter protein. Renilla luciferase/coelenterazine has also been used as the bioluminescence donor in bioluminescence resonance energy transfer (BRET) to study protein-protein interactions.</p> <p>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 peroxyxynitrite in cells or tissues. (Find more information under „Applications“).</p>
Quantity	50 μg
Excitation / Emission Maxima	$\lambda_{\text{ex}} \backslash \lambda_{\text{em}} = 429/466 \text{ nm}$; Extinction coefficient: ca. 7400 at 427 nm (for 1 mg/mL concentration diluted by 1:50 ratio in 50 mM sodium phosphate buffer with pH 7.0). The molar extinction coefficient can be used to determine the concentration of a coelenterazine stock solution. In aqueous solution, the extinction coefficient is ca. 7400 $\text{cm}^{-1}\text{M}^{-1}$ at 427 nm for a coelenterazine stock solution of 1mg/mL concentration diluted by 1:50 ratio in 50 mM sodium phosphate buffer (pH 7.0).
Molecular Structure	
Molecular Weight / Molecular Formula	423.5 Da; $\text{C}_{26}\text{H}_{21}\text{N}_3\text{O}_3$
Purity	>98% (as determined by HPLC)
Appearance / Formulation / Solubility	Yellow solid. Coelenterazine and the derivatives can be reconstituted by dissolving in methanol or ethanol (prepare a stock solution >1 mg/ml). The stock solution can be further diluted with buffer to the appropriate working concentration. We recommend fresh working concentration be prepared each time to avoid precipitate and decomposition. Do not dissolve in dimethylsulfoxide (DMSO) as coelenterazine may be unstable in this solvent. Hardly soluble in water.
Storage & Stability	Store at -20°C . Store in tightly sealed vial. Protect from light. Solution is susceptible to oxidation by air. For best results, keep solution from light and store at $<-70^\circ\text{C}$ 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	<ol style="list-style-type: none">1) Meth. Cell Biol. 40, 305(1994)2) Meth. Enzymol. 172, 164(1989)3) J. Cell Biol. 115, 1259(1991)4) Cell Calcium, 14, 373 (1993)5) Proc. Natl. Acad. Sci. USA 96, 151(1999)6) Free Radic. Biol. Med. 28, 1232(2000)7) Circ. Res. 84, 1203(1999)8) Immunol. Today 15, 7(1994)9) Anal. Biochem. 206, 273(1992)10) Biochem. Biophys. Res. Commun. 233, 349(1997)

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 peroxyxynitrite in cells and tissues. Thus, coelenterazine is also used for chemiluminescent detection of superoxide anions and peroxyxynitrite 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 μ M to >100 μ M. 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	$\lambda_{em}(nm)$	Total Light (%)	Initial Intensity (%)
native	475	100	45
400a	400		
<i>cp</i>	470	23	135
<i>e</i>	418, 475	137	900
<i>f</i>	473	28	45
<i>h</i>	475	41	135
<i>n</i>	475	47	900

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

Analog	$\lambda_{em}(nm)$	Relative luminescence capacity	Relative intensity	Half-rise time (s)
native	465	1.0	1.00	0.4-0.8
<i>cp</i>	442	0.95	15	0.15-0.3
<i>e</i>	405, 465	0.50	4	0.15-0.3
<i>f</i>	473	0.80	18	0.4-0.8
<i>fcp</i>	452	0.57	135	0.4-0.8
<i>h</i>	475	0.82	10	0.4-0.8
<i>hcp</i>	444	0.67	190	0.15-0.3
<i>i</i>	476	0.70	0.03	8
<i>ip</i>	441	0.54	47	1
<i>n</i>	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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