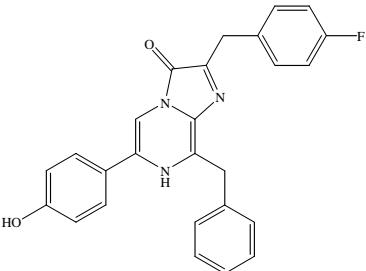


Instruction Manual

Catalog Number	PK-CA707-10114
Description	Coelenterazine <i>f</i> is a synthetic derivative of coelenterazine. The luminescence intensity of its aequorin complex is almost 20 times higher than that of the aequorin formed from native coelenterazine while its emission maximum is about 8 nm longer than that of the latter. It is a highly sensitive and specific intracellular luminophore that is useful for measuring changes in superoxide and Ca ²⁺ levels in cells transfected with apoaequorin cDNA. 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“).
Quantity	50 µg
Excitation / Emission Maxima	$\lambda_{ex} \backslash \lambda_{em} = 437/473 \text{ nm}$; Extinction coefficient: 8,700 (in MeOH)
Molecular Structure	 The chemical structure of Coelenterazine f is a complex heterocyclic molecule. It features a central benzimidazole ring system. One nitrogen atom of the benzimidazole is substituted with a 4-hydroxyphenyl group. The other nitrogen atom is substituted with a 4-fluorophenyl group. The 2-position of the benzimidazole ring is substituted with a 4-phenylbutyl group. The 5-position of the benzimidazole ring is substituted with a 4-phenylbutyl group. The 6-position of the benzimidazole ring is substituted with a 4-phenylbutyl group.
Molecular Weight / Molecular Formula	425.46 Da; C ₂₆ H ₂₀ FN ₃ O ₂
Purity	>95% (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 <i>f</i> may be unstable in this solvent. Low solubility 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°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) <i>Biochem. J.</i> 261 , 913(1989) 2) <i>Cell Calcium</i> 12 , 635(1991) 3) <i>Cell Calcium</i> , 14 , 373 (1993); 4) <i>Mol Imaging</i> , 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 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 Ca²⁺ binds to the complex. The luminescence intensity is correlated to the Ca²⁺ 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	λ _{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	λ _{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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